09/758036
Aff # 13

=> s potassium
L1 933730 POTASSIUM

=> s channel

L2 807406 CHANNEL

=> s blocker? or inhibit? or block or blocks or activat? or agonist? or antagonist?
2 FILES SEARCHED...
L3 7964570 BLOCKER? OR INHIBIT? OR BLOCK OR BLOCKS OR ACTIVAT? OR AGONIST?
OR ANTAGONIST?

=> s cerevisiae

L4 201869 CEREVISIAE

=> s l1 and l2 and l3 and l4

L5 155 L1 AND L2 AND L3 AND L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 73 DUP REM L5 (82 DUPLICATES REMOVED)

=> s l6 and py<2000

1 FILES SEARCHED...
3 FILES SEARCHED...
4 FILES SEARCHED...
L7 55 L6 AND PY<2000

=> d l7 ibib abs 1-55

L7 ANSWER 1 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:61230 BIOSIS
DOCUMENT NUMBER: PREV200000061230
TITLE: K+-dependent composite gating of the yeast K+ ***channel*** , TOK1.
AUTHOR(S): Loukin, Stephen H. (1); Saimi, Yoshiro
CORPORATE SOURCE: (1) Laboratory of Molecular Biology, University of
Wisconsin, 1525 Linden Dr., Madison, WI USA
SOURCE: Biophysical Journal, (***Dec., 1999***) Vol. 77, No. 6,
pp. 3060-3070.
ISSN: 0006-3495.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB TOK1 encodes an outwardly rectifying K+ ***channel*** in the plasma
membrane of the budding yeast Saccharomyces ***cerevisiae*** . It is capable of dwelling in two kinetically distinct impermeable states, a near-instantaneously ***activating*** R state and a set of related delayed ***activating*** C states (formerly called C2 and C1, respectively). Dwell in the R state is dependent on membrane potential and
both internal and external K+ in a manner consistent with the K+ electrochemical potential being its determinant, where dwell in the C states is dependent on voltage and only external K+. Whereas ***activation*** from the C states showed high temperature dependencies,
typical of gating transitions in other Shaker-like channels, ***activation*** from the R state had a temperature dependence nearly as
low as that of simple ionic diffusion. These findings lead us to conclude that although the C states reflect the activity of an internally oriented ***channel*** gate, the R state results from an intrinsic gating property of the ***channel*** filter region.

L7 ANSWER 2 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:2530 BIOSIS
DOCUMENT NUMBER: PREV200000002530
TITLE: A molecular target for viral killer toxin: TOK1 ***potassium*** channels.
AUTHOR(S): Ahmed, Aamir; Sesti, Federico; Ilan, Nitza; Shih, Theodore
M.; Sturley, Stephen L.; Goldstein, Steve A. N. (1)
CORPORATE SOURCE: (1) Departments of Pediatrics and Cellular and Molecular
Physiology, Boyer Center for Molecular Medicine Yale University School of Medicine, New Haven, CT, 06536 USA
SOURCE: Cell, (***Oct. 29, 1999***) Vol. 99, No. 3, pp. 283-291.
ISSN: 0092-8674.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Killer strains of S. ***cerevisiae*** harbor double-stranded RNA viruses and secrete protein toxins that kill virus-free cells. The K1 killer toxin acts on sensitive yeast cells to perturb ***potassium*** homeostasis and cause cell death. Here, the toxin is shown to ***activate*** the plasma membrane ***potassium*** ***channel***
of S. ***cerevisiae*** ; TOK1. Genetic deletion of TOK1 confers toxin resistance; overexpression increases susceptibility. Cells expressing TOK1 exhibit toxin-induced ***potassium*** flux; those without the gene do not. K1 toxin acts in the absence of other viral or yeast products: toxin synthesized from a cDNA increases open probability of single TOK1 channels
(via reversible destabilization of closed states) whether channels are studied in yeast cells or X. laevis oocytes.

L7 ANSWER 3 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:484291 BIOSIS
DOCUMENT NUMBER: PREV199900484291
TITLE: Mutations in the yeast two pore K+ ***channel*** YKC1 identify functional differences between the pore domains.
AUTHOR(S): Vergani, Paola; Blatt, Michael R. (1)
CORPORATE SOURCE: (1) Laboratory of Plant Physiology and Biophysics,
University of London, London UK
SOURCE: FEBS Letters, (***Sept. 24, 1999***) Vol. 458, No. 3, pp. 285-291.
ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The K+ ***channel*** of Saccharomyces ***cerevisiae*** encoded by
the YKC1 gene includes two pore-loop sequences that are thought to form the hydrophilic lining of the pore. Gating of the ***channel*** is promoted by membrane depolarisation and is regulated by the extracellular K+ concentration ((K+)o) both in the yeast and when expressed in Xenopus
oocytes. Our previous work showed that substitutions of equivalent residues L293 and A428 within the pore-loops had qualitatively similar effects on both the (K+)o-sensitivity of ***channel*** gating and its voltage-dependence. Here, we report that mutations of equivalent residues N275 and N410, N-terminal from the K+ ***channel*** signature sequences of the two pores, have very different actions on ***channel***
gating and, in this case, are without effect on its voltage-sensitivity. The mutation N410D slowed current ***activation*** in a (K+)o-dependent manner and it accelerated deactivation, but without significant effect on the apparent affinity for K+. The N275D mutant, by contrast, had little effect on the (K+)o-sensitivity for ***activation*** and it greatly altered the (K+)o-dependence of current deactivation. Neither mutant affected the voltage-dependence of the steady-state current nor the ability for other alkali cations to substitute for K+ in regulating gating. The double mutant N410D-N275D showed characteristics of N410D in the (K+)o-sensitivity of current ***activation*** and of N275D in the (K+)o-sensitivity of deactivation, suggesting that little interaction occurs between pore domains with mutations at these sites. The results indicate that the two pore domains are not functionally equivalent and they suggest that the regulation of gating by external K+ is mediated by K+ binding at two physically distinct sites with different actions.

L7 ANSWER 4 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1999:443095 BIOSIS
DOCUMENT NUMBER: PREV199900443095
TITLE: Overexpression of the volatile anesthetic- ***activated*** baseline K+ ***channel*** TOK1 ***inhibits*** yeast growth.
AUTHOR(S): Yost, Charles S. (1); O'Rourke, Sean M. (1); Sampson, Elizabeth R. (1); Herskowitz, Ira (1)
CORPORATE SOURCE: (1) University of California San Francisco, San Francisco, CA USA
SOURCE: Anesthesiology (Hagerstown), (***Sept., 1999***) Vol. 91, No. 3A, pp. A355.
Meeting Info.: Annual Meeting of the American Society of Anesthesiologists Dallas, Texas, USA October 9-13, 1999 American Society of Anesthesiologists
. ISSN: 0003-3022.
DOCUMENT TYPE: Conference
LANGUAGE: English

L7 ANSWER 5 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:397619 BIOSIS
DOCUMENT NUMBER: PREV199900397619
TITLE: Calcium- ***inhibited*** non-selective cation "channels" (Nsc1) admit large inward currents through the yeast plasma membrane.
AUTHOR(S): Bihler, H. (1); Slayman, C. L. (1); Bertl, A.
CORPORATE SOURCE: (1) School of Medicine, Yale University, New Haven, CT USA
SOURCE: Folia Microbiologica, (1999) Vol. 44, No. 2, pp. 221-222.
Meeting Info.: 16th Small Meeting on Yeast Transport and Energetics Casta-Papiernicka, Slovakia September 23-27, 1998
ISSN: 0015-5632.
DOCUMENT TYPE: Conference
LANGUAGE: English

L7 ANSWER 6 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:397553 BIOSIS
DOCUMENT NUMBER: PREV199900397553
TITLE: Genetic selection of inward-rectifying K+ ***channel*** mutants with reduced Cs+ sensitivity by random recombinant DNA shuffling mutagenesis and mutant selection in yeast.
AUTHOR(S): Ichida, Audrey M.; Baizabal-Aguirre, Victor M.; Schroeder, Julian I. (1)
CORPORATE SOURCE: (1) Department of Biology and Center for Molecular Genetics, University of California at San Diego, La Jolla, CA, 92093-0116 USA
SOURCE: Journal of Experimental Botany, (***June, 1999***) Vol. 50, No. SPEC. ISS., pp. 967-978.
ISSN: 0022-0957.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Structure-function relationships of voltage-dependent ion channels have been analysed by site-directed mutagenesis and functional electrophysiological characterizations. A complementary genetic selection approach for identifying interesting K+ ***channel*** mutations, allowing selection from a large random pool of K+ ***channel*** mutants, is described here. Non-inactivating inward-rectifying ***potassium*** channels provide an important mechanism for K+ uptake into plant cells. The Arabidopsis Kin+ ***channel*** , KAT1, functionally complements a yeast strain deficient in K+ uptake. The alkali metal Cs+ ***blocks*** Kin+ channels and ***inhibits*** growth of yeast cells expressing KAT1. In this study a mutagenesis method called 'DNA shuffling' (or 'recombinant PCR') was applied to generate random mutants in the KAT1 ***channel*** . Randomly mutated libraries of KAT1 were expressed in yeast and Cs+-resistant colonies were selected. KAT1 mutants that conferred a Cs+-resistant phenotype for yeast growth were functionally characterized by expression in Xenopus oocytes and two electrode voltage clamp analysis. K+ ***channel*** properties, such as Cs+- ***block*** sensitivity, cation selectivity, and steady-state ***activation*** were altered by mutating amino acids in the pore region, but also in regions adjacent to the pore region of the KAT1 ***channel*** . Amino acid substitutions previously not targeted for site-directed mutagenesis were identified that affect Cs+ ***block*** of K+ channels. Two amino acid positions, I209 in the S5 domain and E269, were mutated in more than one of the Cs+-resistant mutants indicating important roles in Cs+ sensitivity. Shifts in steady-state ***activation*** and/or resistance to ***block*** by Cs+ were determined to be mechanisms which contribute to the Cs+ resistance of the selected mutants.

L7 ANSWER 7 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:212609 BIOSIS
DOCUMENT NUMBER: PREV199900212609
TITLE: ***Potassium*** uptake through the TOK1 K+ ***channel*** in the budding yeast.
AUTHOR(S): Fairman, C.; Zhou, X.-L.; Kung, C. (1)
CORPORATE SOURCE: (1) Laboratory of Molecular Biology, University of Wisconsin, 1525 Linden Dr., Madison, WI, 53706 USA
SOURCE: Journal of Membrane Biology, (***March 15, 1999***) Vol. 168, No. 2, pp. 149-157.
ISSN: 0022-2631.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The current through TOK1 (YKC1), the outward-rectifying K+ ***channel*** in Saccharomyces ***cerevisiae*** , was amplified by expressing TOK1 from a plasmid driven by a strong constitutive promoter. TOK1 so hyper-expressed could overcome the K+ auxotrophy of a mutant missing the two K+ transporters, TRK1 and TRK2. This trk1DELTA trk2DELTA double mutant hyperexpressing the TOK1 transgene had a higher internal K+ content than one expressing the empty plasmid. We examined protoplasts of these TOK1-hyperexpressing cells under a patch clamp. Besides the expected K+ outward current ***activating*** at membrane potential (Vm) above the K+ equilibrium potential (EK+), a small inward current was consistently observed when the Vm was slightly below EK+. The inward and the outward currents are similar in their ***activation*** rates, deactivation rates, ion specificities and Ba2+ ***inhibition*** , indicating that they flow through the same ***channel*** . Thus, the yeast outwardly rectifying K+ ***channel*** can take up K+ into yeast cells, at least under certain conditions.

L7 ANSWER 8 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:57903 BIOSIS
DOCUMENT NUMBER: PREV199900057903
TITLE: Mutations in the pore regions of the yeast K+ ***channel*** YKC1 affect gating by extracellular K.
AUTHOR(S): Vergani, Paola (1); Hamilton, David; Jarvis, Simon; Blatt, Michael R.
CORPORATE SOURCE: (1) Lab. Plant Physiol. Biophysics, Univ. London, Wye Coll., Wye, Kent TN25 5AH UK
SOURCE: EMBO (European Molecular Biology Organization) Journal, (***Dec. 15, 1998***) Vol. 17, No. 24, pp. 7190-7198.
ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The product of the Saccharomyces ***cerevisiae*** K+-***channel*** gene YKC1 includes two pore-loop sequences that are thought to form the hydrophilic lining of the pore. Gating of the ***channel*** is promoted by membrane depolarization and is regulated by extracellular K+

concentration ((K+)o) both in the yeast and when expressed in Xenopus oocytes. Analysis of the wild-type current now shows that: (i) (K+)o suppresses a very slowly relaxing component, accelerating ***activation*** ; (ii) (K+)o slows deactivation in a dose-dependent fashion; and (iii) Rb+, Cs+ and, to a lesser extent, Na+ substitute for K+ in its action on gating. We have identified single residues, L293 and A428, at equivalent positions within the two pore loops that affect the (K+)o sensitivity. Substitution of these residues gave channels with reduced sensitivity to (K+)o in macroscopic current kinetics and voltage dependence, but had only minor effects on selectivity among alkali cations in gating and on single- ***channel*** conductance. In some mutants, ***activation*** was slowed sufficiently to confer a sigmoidicity to current rise at low (K+)o. The results indicate that these residues are involved in (K+)o sensing. Their situation close to the permeation pathway points to an interaction between gating and permeation.

L7 ANSWER 9 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:388702 BIOSIS
DOCUMENT NUMBER: PREV199800388702
TITLE: NSC1: A novel high-current inward rectifier for cations in the plasma membrane of Saccharomyces ***cerevisiae*** .
AUTHOR(S): Bihler, Hermann; Slayman, Clifford L.; Bertl, Adam (1)
CORPORATE SOURCE: (1) Bot. Inst. I, Univ. Karlsruhe, Kaiserstr. 2, D-76128 Karlsruhe Germany
SOURCE: FEBS Letters, (***July 31, 1998***) Vol. 432, No. 1-2, pp. 59-64.
ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The plasma membrane of the yeast Saccharomyces ***cerevisiae*** possesses a non-specific cation ' ***channel*** ', tentatively dubbed NSC1, which is blocked by normal (mM) calcium and other divalent metal ions, is unblocked by reduction of extracellular free divalents below apprx10 muM, and is independent of the identified ***potassium*** ***channel*** and porters in yeast, Duk1p, Trk1p, and Trk2p. Ion currents through NSC1, observed by means of whole-cell patch recording, have the following characteristics: Large amplitude, often exceeding 1 nA of K+/cell at -200 mV, in tetraploid yeast, sufficient to double the normal intracellular K+ concentration within 10 s; non-saturation at large negative voltages; complicated ***activation*** kinetics, in which apprx50% of the total current arises nearly instantaneously with a voltage-clamp step, while the remainder develops as two components, with time constants of apprx100 ms and apprx1.3 s; and voltage independence of both the ***activation*** time constants and the associated fractional current amplitudes.

L7 ANSWER 10 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:224591 BIOSIS
DOCUMENT NUMBER: PREV199800224591
TITLE: Investigation of the yeast mitochondrial unselective ***channel*** in intact permeablized spheroplasts.
AUTHOR(S): Manon, Stephen (1); Guerin, Martine
CORPORATE SOURCE: (1) Institut Biochimie Genetique Cellulaires Centre National Recherche Scientifique, Univ. Victor Segalen-Bordeaux II, 1 rue Camille Saint-Saens, F-33077 Bordeaux cedex France
SOURCE: Biochemistry and Molecular Biology International, (***March, 1998***) Vol. 44, No. 3, pp. 565-575.
ISSN: 1039-9712.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The existence of an activity corresponding to the nucleotide-induced Yeast Mitochondria Unselective ***Channel*** (YMUC2) of isolated mitochondria was investigated in permeabilized and intact spheroplasts of the baker's yeast Yeast Foam. In nystatin-permeabilized spheroplasts, ATP and GDP-beta-S induced a decavanadate-sensitive stimulation of the respiration only under conditions equivalent to those previously reported for isolated mitochondria (low phosphate concentration, presence of a salt). On intact spheroplasts. parallel measurements of respiration rate, (ATP)/(ADP) ratio and mitochondrial transmembrane potential allowed to show that the addition of the glucose analog 2-deoxyglucose decreased the permeability of the inner mitochondrial membrane owing to cellular ATP depletion. This strongly supports the hypothesis that Yeast Mitochondria Unspecific ***Channel*** is active in situ and ***inhibited*** by cellular (ATP) depletion.

L7 ANSWER 11 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:175813 BIOSIS
DOCUMENT NUMBER: PREV199800175813
TITLE: Physiological characterization of the yeast plasma membrane outward rectifying K+ ***channel*** , DUK1 (TOK1), in situ.
AUTHOR(S): Bertl, A. (1); Bihler, H.; Reid, J. D.; Kettner, C.; Slayman, C. L.
CORPORATE SOURCE: (1) Botanisches Institut I, Universitaet Karlsruhe, Kaiserstrasse 2, D-76128 Karlsruhe Germany
SOURCE: Journal of Membrane Biology, (***March 1, 1998***) Vol. 162, No. 1, pp. 67-80.
ISSN: 0022-2631.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The major voltage-dependent ion ***channel*** in the plasma membrane of Saccharomyces ***cerevisiae*** , a conspicuous outwardly rectifying K+ ***channel*** , was first dubbed YPK1 and later renamed according to its registered gene names (DUK1, TOK1). It has proven novel in both structure and function. Whole-cell patch-clamp studies of the ***channel*** directly on yeast protoplasts now extend our earlier description obtained from isolated patches of yeast membrane (Bertl & Slayman, 1992; Bertl et al., 1993), and provide new data both on the contributions of ***channel*** properties to yeast physiology and on possible contributions of molecular structure to ***channel*** properties. Three recording tactics produce completely equivalent results and thereby allow great flexibility in the design of experiments: whole-cell voltage clamp with sustained voltage steps (apprx2.5 sec), whole-cell voltage clamp with slow voltage ramps (5 sec, -40 to +100 mV), and time-averaging of single- ***channel*** currents. ***Activation*** of Duk1 channels under steady-state conditions is dependent upon ATP in the cytoplasmic solution, and the absence of ATP results in ***channel*** "rundown"-decreasing numbers of activable channels over periods of 10 min to 1 hr from the start of patch recording. Several putative serine- and threonine-phosphorylation sites, as well as a variant ATP-binding fold, exist in the molecule as potential mediators of the ATP effects. The ***channel*** runs down similarly following cytoplasmic acidification, but is almost completely insensitive to extracellular pH changes (8.0 to 5.5 tested). This remarkable asymmetry may depend on the protein's strongly asymmetric distribution of histidine residues, with 10 out of 12 predicted to lie close to the membrane-cytoplasm interface. Further data confirm the well-recognized observation that changes of K+ concentration, intracellular or extracellular, can shift the gating voltage of Duk1p in the direction of EK. Among the other alkali-metal cations tested, extracellular Rb+ and Cs+-but not Na+substitute almost completely for K+. Extracellular TEA+ ***inhibits*** whole-cell K+ currents through Duk1p with a KI of 2.8 mM, and does so probably by reducing the single- ***channel*** current.

L7 ANSWER 12 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:506676 BIOSIS
DOCUMENT NUMBER: PREV199799805879
TITLE: Palytoxin-induced ***channel*** formation with the Na+/K+-ATPase does not require a catalytically active enzyme.
AUTHOR(S): Scheiner-Bobis, Georgios (1); Schneider, Heike
CORPORATE SOURCE: (1) Inst. Biochemie Endokrinologie, Fachbereich Veterinaermediin, Justus-Liebig-Univ. Giessen, Frankfurter Strasse 100, D-35392 Giessen Germany
SOURCE: European Journal of Biochemistry, (1997) Vol. 248, No. 3, pp. 717-723.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English

AB It has been demonstrated that palytoxin binds to and forms a ***channel*** within the Na+/K+-ATPase. To investigate whether palytoxin-induced ***channel*** formation within the sodium pump can occur independently of ATP hydrolysis and phosphorylation of the enzyme, an Asp369 fwdarw Ala mutant of the alpha-1 subunit of the sheep sodium pump was produced and coexpressed with beta subunits in the yeast Saccharomyces ***cerevisiae*** . This aspartic acid residue, which during ion transport becomes phosphorylated from ATP, is essential for the function of the sodium pump. Therefore, as expected, microsomes isolated from yeast expressing the mutant sodium pump do not exhibit any ouabain sensitive ATPase activity, whereas in microsomes from yeast expressing the wild-type sodium pump, 60% of the total ATPase activity is ouabain-sensitive. Ouabain binds to yeast membranes containing either wild-type or mutant sodium pumps with similar B-max (1.45 +- 0.05 versus 1.37 +- 0.02 pmol/mg) and K-d values (27.7 +- 0.91 versus 29.57 +- 0.93 nM), thus indicating that the mutant sodium pumps are expressed in the yeast and that the mutation does not considerably affect the conformation of the enzyme. In the presence of phosphate ouabain binds to microsomes containing the wild-type sodium pump with a K-d of 3.62 +- 0.34 nM, showing that, although not necessary, phosphoenzyme formation enhances binding of the steroid. Phosphate or ATP, however, ***inhibit*** binding of ouabain to microsomes containing the mutant sodium pump with IC-50 values of 78 +- 3 mu-M and 3.0 +- 0.4 mu-M, respectively. Despite these radical changes in the interactions of the mutant enzyme with ouabain, the interactions with palytoxin are not affected by the mutation. Palytoxin causes K+ efflux from yeast cells expressing the wild-type or mutant sodium pumps with EC-50 values of 3.5 +- 0.4 nM and 6.2 +- 0.9 nM, respectively. Palytoxin-induced efflux from cells expressing wild-type or mutant sodium pumps occurs with similar t-1/2 values of 20.3 +- 2.1 min and 22.2 +- 3.1 min, respectively. Ouabain ***inhibits*** K+ efflux from both cell types with IC-50 values of 28 +- 2 mu-M and 210 +- 15 mu-M, respectively. Cells expressing the Asp369 fwdarw Ala mutants have an IC-50 7.5-fold higher than that obtained with cells expressing the wild-type sodium pumps, possibly because ATP or phosphate present in the cytosol of the yeast cells influence and decrease ouabain binding to the mutant sodium pump. Thus, while ouabain binding and the associated ***inhibition*** of ion fluxes is promoted by phosphorylation of the wild-type enzyme by phosphate or ATP, palytoxin-induced ***channel*** formation is independent of phosphorylation and can be separated from the ATPase function of the sodium pump. Since ion fluxes through the sodium pump protein do not depend on ATP hydrolysis, the results suggest that the ionophores of pumps and ion channels might share common structural features.

L7 ANSWER 13 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:435978 BIOSIS
DOCUMENT NUMBER: PREV199799735181
TITLE: Random mutagenesis reveals a region important for gating of the yeast K+ ***channel*** Ykc1.
AUTHOR(S): Loukin, Stephen H.; Vaillant, Brian; Zhou, Xin-Liang; Spalding, Edgar P.; Kung, Ching; Saimi, Yoshiro (1)
CORPORATE SOURCE: (1) Lab. Mol. Biol., Univ. Wisconsin, Madison, WI 53706 USA
SOURCE: EMBO (European Molecular Biology Organization) Journal, (1997) Vol. 16, No. 16, pp. 4817-4825.
ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
AB YKC1 (TOK1, DUK1, YORK) encodes the outwardly rectifying K+ ***channel*** of the yeast plasma membrane. Non-targeted mutations of YKC1 were isolated by their ability to completely ***block*** proliferation when expressed in yeast. All such mutations examined occurred near the cytoplasmic ends of the transmembrane segments following either of the duplicated P loops, which we termed the 'post-P loop' (PP) regions. These PP mutations specifically caused marked defects in the 'C-1' states, a set of interrelated closed states that Ykc1 enters and exits at rates of tens to hundreds of milliseconds. These results indicate that the Ykc1 PP region plays a role in determining closed state conformations and that non-targeted mutagenesis and microbial selection can be a valuable tool for probing structure-function relationships of ion channels.

L7 ANSWER 14 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:423905 BIOSIS
DOCUMENT NUMBER: PREV199799723108
TITLE: ***Channel*** functions in the yeast Saccharomyces ***cerevisiae*** : DUK1, the major outward rectifier for ***potassium*** .
AUTHOR(S): Bertl, A.; Slayman, C. L.
CORPORATE SOURCE: Dep. Cellular Molecular Physiol., Yale Sch. Med., New Haven, CT USA
SOURCE: Wiener Klinische Wochenschrift, (1997) Vol. 109, No. 12-13, pp. 512.
Meeting Info.: Symposium on Renal Electrolyte Metabolism: Physiology and Pathophysiology Vienna, Austria June 28, 1997
ISSN: 0043-5325.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

L7 ANSWER 15 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:392459 BIOSIS
DOCUMENT NUMBER: PREV199799691662
TITLE: Antimycotic activity of lysozyme and its contribution to antifungal humoral defence reactions in Galleria mellonella.
AUTHOR(S): Vilcinskas, Andreas (1); Matha, Vladimir
CORPORATE SOURCE: (1) Inst. Zool., Free Univ. Berlin, Koenig-Luise-Str. 1-03, 14195 Berin Germany
SOURCE: Animal Biology, (1997) Vol. 6, No. 1, pp. 19-29.
ISSN: 1121-1431.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Preinjection with zymosan or heat-inactivated yeast cells Sacharomyces ***cerevisiae*** increased the survival rates of Galleria mellonella larvae after following injection of normally lethal dosages of living yeasts. Immunized larvae showed no increased survival rates after infection with blastospores of the entomopathogen Beavueria bassiana but survived longer than untreated larvae. Cell-free hemolymphy, obtained from immunized larvae, ***inhibited*** the growth of yeasts in liquid cultures stronger than that of untreated larvae whereas their influence on blastospore germination of B. bassiana was not significantly different. A remarkable increase in antibacterial activity within the hemolymph was detected after injection of fungal provocators like zymosan or fungal cells. Protein pattern in SDS-PAGE of hemolymph from larvae immunized with bacterial or fungal provocators exhibited similarity concerning new or enhanced protein bands, suggesting an unspecific induction of protein synthesis. Lysozyme purified from cell-free hemolymph of G. mellonella larvae ***inhibited*** the growth of yeasts in vitro. Hen egg-white lysozyme, human lysozyme and cecropins purified from Hyalophora cecropia also showed this ***inhibiting*** effect. Hen egg-white lysozyme also ***inhibited*** growth and affected the lyphae formation of the non-pathogenic saprophyte Absidia glauca but caused no effects on the growth of virulent strains of B. bassiana or M. anisopliae. The antifungal activity of lysozyme against yeast in vitro was not caused by chitinolytic activity but influenced by nutrition and ***potassium*** concentration in the medium. The antifungal effect of lysozyme was neutralized depending on the dose by increased levels of ***potassium*** in the medium or by presence of the ***potassium*** ***channel*** ***blocker*** tetraethylammoniumchloride. The contribution of lysozyme in antifungal

immunity in insects is discussed with regard to its observed effects on fungal cells.

L7 ANSWER 16 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:384674 BIOSIS
DOCUMENT NUMBER: PREV199799683877
TITLE: Mammalian gene encoding growth factor- ***activated*** cation ***channel*** is homologue to yeast microsomal protein Sec62 and maps to human chromosome 3.
AUTHOR(S): Fantino, E.; Church, D.; Bengtsson, U.; Gargus, J. J.
CORPORATE SOURCE: Univ. California, Irvine, CA USA
SOURCE: Journal of General Physiology, (1997) Vol. 110, No. 1, pp.
44A.
Meeting Info.: Fifty-First Annual Meeting of the Society of General Physiologists Woods Hole, Massachusetts, USA September 4-6, 1997
ISSN: 0022-1295.
DOCUMENT TYPE: Conference; Abstract; Conference
LANGUAGE: English

L7 ANSWER 17 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:292858 BIOSIS
DOCUMENT NUMBER: PREV199799592061
TITLE: Functional comparison of plant inward-rectifier channels expressed in yeast.
AUTHOR(S): Bertl, Adam (1); Reid, John D.; Sentenac, Herve; Slayman, Clifford L.
CORPORATE SOURCE: (1) Botanisches Inst. I, Univ. Karlsruhe, Kaiserstrasse 2, D-76128 Karlsruhe Germany
SOURCE: Journal of Experimental Botany, (1997) Vol. 48, No. SPEC. ISSUE, pp. 405-413.
ISSN: 0022-0957.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Functional expression of plant ion channels in the yeast Saccharomyces ***cerevisiae*** is readily demonstrated by the successful screening of plant cDNA libraries for complementation of transport defects in especially constructed strains of yeast. The first experiments of this sort identified two ***potassium*** - ***channel*** genes from Arabidopsis thaliana, designated KAT1 and AKT1 (Anderson et al., 1992; Sentenac et al., 1992), both of which code for proteins resembling the Shaker superfamily of K+ channels in animal cells. Patch-clamp analysis, directly in yeast, of the two ***channel*** proteins (Kat1 and Akt1) reveals both functional similarities and functional differences: similarities in selectivity and in normal gating kinetics; and differences in time-dependent effects of ion replacement, in the affinities of blocking ions, and in dependence of gating kinetics on extracellular K+. Kat1, previously described in yeast (Bertl et al., 1995), is about 20-fold more permeable to K+ than to Na+ or NH-4+, shows K+-independent gating kinetics, and is blocked with moderate effectiveness (30-50% at 10 mM) by barium and tetraethylammonium (TEA+) ions. Akt1, by contrast, is weakly ***inhibited*** by TEA+, more strongly ***inhibited*** by Ba-2+, and very strongly ***inhibited*** by Cs+. Furthermore Na+ and NH-4+, while having about the same permeance to Akt1 as to Kat1, have delayed effects on Akt1: brief replacement of extracellular K+ by Na+ enhances by nearly 100% the subsequent K+ currents after sodium removal; and brief replacement of K+ by NH-4+ reduces subsequent K+ currents by nearly 75%. Furthermore, lowering of extracellular K+ concentration, by replacement with osmotically equivalent sorbitol, significantly retards the opening of Akt1 channels; that is, the gating kinetics for Akt1 are clearly influenced by the concentration of permeant ions. In this respect, Akt1 resembles the native yeast outward rectifier, Ypk1 (Duk1; Reid et al., 1996). The data suggest that all of the ions tested bind within the open channels, such that the weakly permeant species (Na+, NH-4+) are easily displaced by K+, but the blocking species (Cs+, Ba-2+, TEA+) are not easily displaced. With Akt1, furthermore, the permeant ions bind to a modulator site where they persist after removal from the medium, and through which they can alter the ***channel*** conductance. Extracellular K+ itself also binds to a modulator site, thereby enhancing the rate of opening of Akt1.

L7 ANSWER 18 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:292829 BIOSIS
DOCUMENT NUMBER: PREV199799592032
TITLE: Ion channels in guard cells of Arabidopsis thaliana (L.) Heynh.
AUTHOR(S): Roelfsema, M. Rob G.; Prins, Hidde B. A.
CORPORATE SOURCE: Lab. Plant Physiol., Dep. Plant Biol., Univ. Groningen, PO Box 14, NL-9750 AA Haren Netherlands
SOURCE: Planta (Heidelberg), (1997) Vol. 202, No. 1, pp. 18-27.
ISSN: 0032-0935.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Despite the availability of many mutants for signal transduction, Arabidopsis thaliana guard cells have so far not been used in electrophysiological research. Problems with the isolation of epidermal strips and the small size of A. thaliana guard cells were often prohibiting. In the present study these difficulties were overcome and guard cells were impaled with double-barreled microelectrodes. Membrane-potential recordings were often stable for over half an hour and voltage-clamp measurements could be conducted. The guard cells were found to exhibit two states. The majority of the guard cells had depolarized membrane potentials, which were largely dependent on external K+ concentrations. Other cells displayed spontaneous transitions to a more hyperpolarized state, at which the free-running membrane potential (E-m) was not sensitive to the external K+ concentration. Two outward-rectifying conductances were identified in cells in the depolarized state. A slow outward-rectifying ***channel*** (s-ORC) had properties resembling the K+-selective ORC of Vicia faba guard cells (Blatt, 1988, J Membr Biol 102: 235-246). The ***activation*** and inactivation times and the ***activation*** potential, all depended on the reversal potential (E-rev) of the s-ORC conductance. The s-ORC was blocked by Ba-2+ (K-1/2 = 0.3-1.3 mM) and verapamil (K-1/2 = 15-20 mu-M). A second rapid outward-rectifying conductance (r-ORC) ***activated*** instantaneously upon stepping the voltage to positive values and was stimulated by Ba-2+. Inward-rectifying channels (IRC) were only observed in cells in the hyperpolarized state. The ***activation*** time and ***activation*** potential of this ***channel*** were not sensitive to the external K+ concentration. The slow ***activation*** of the IRC (t-1/2 apprxeq 0.5 s) and its negative ***activation*** potential (V-threshold = -155 mV) resemble the values found for the KAT1 ***channel*** expressed in Saccharomyces ***cerevisiae*** (Bertl et al., 1995, Proc Natl Acad Sci USA 92: 2701-2705). The results indicate that A. thaliana guard cells provide an excellent system for the study of signal transduction processes.

L7 ANSWER 19 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:221702 BIOSIS
DOCUMENT NUMBER: PREV199799513418
TITLE: Extracellular K+ and Ba-2+ mediate voltage-dependent inactivation of the outward-rectifying K+ ***channel*** encoded by the yeast gene TOK1.
AUTHOR(S): Vergani, Paola; Miosga, Thomas; Jarvis, Simon M.; Blatt, Michael R. (1)
CORPORATE SOURCE: (1) Lab. Plant Physiol. Biophysics, Wye Coll., Univ. London, Wye, Kent TN25 5AH UK
SOURCE: FEBS Letters, (1997) Vol. 405, No. 3, pp. 337-344.
ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Gating of the yeast K+ ***channel*** encoded by the Saccharomyces ***cerevisiae*** gene TOK1, unlike other outward-rectifying K+

channels
that have been cloned, is promoted by membrane voltage (inside positive-going) and repressed by extracellular K+. When expressed in Xenopus laevis oocytes, the TOK1p current rectified strongly outward, its ***activation*** shifting in parallel with the K+ equilibrium potential when the external K+ concentration ((K+)-o) was increased above 3 mM. Analysis of the TOK1p current indicated that two kinetic components contributed to the conductance and the voltage sensitivity of the conductance. By contrast, the (K+)-o sensitivity of the current was accommodated entirely within the slow-relaxing component; it was diminished near 1 mM (K+)-o, and at submillimolar concentrations the voltage dependence of the TOK1-p conductance was insensitive to (K+)-o.
External Rb+, the K+ ***channel*** ***blockers*** Cs+ and Ba-2+ -
but not Na+, Ca-2+ or Mg-2+ - substituted for K+ in control of TOK1p ***activation*** , indicating a specificity in cation interaction with the TOK1p gate. These and additional results indicate that external K+ acts as a ligand to inactivate the TOK1p ***channel*** , and they implicate a gating process mediated by a single cation binding site within the membrane electric field, but distinct from the permeation pathway.

L7 ANSWER 20 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:419233 BIOSIS
DOCUMENT NUMBER: PREV199699141589
TITLE: Changes in voltage ***activation*** , Cs+ sensitivity, and ion permeability in H5 mutants of the plant K+ ***channel*** KAT1.
AUTHOR(S): Becker, Dirk; Dreyer, Ingo; Hoth, Stefan; Reid, John D.; Busch, Heiner; Lehnen, Michaela; Palme, Klaus; Hedrich, Rainer (1)
CORPORATE SOURCE: (1) Inst. Biophysik, Univ. Hannover, Herrenhaeuserstrasse
2, 30419 Hannover Germany
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1996) Vol. 93, No. 15, pp. 8123-8128.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
AB KAT1 is a voltage-dependent inward rectifying K+ ***channel*** cloned
from the higher plant Arabidopsis thaliana (Anderson, J. A., Huprikar, S. S., Kochian, L. V., Lucas, W. J. & Gaber, R. F. (1992) Proc. Natl. Acad. Sci. USA 89, 3736-3740). It is related to the Shaker superfamily of K+ channels characterized by six transmembrane spanning domains (S1-S6) and a
putative pore-forming region between S5 and S6 (H5). The H5 region between
Pro-247 and Pro-271 in KAT1 contains 14 additional amino acids when compared with Shaker (Aldrich, R. W. (1993) Nature (London) 362, 107-108).
We studied various point mutations introduced into H5 to determine whether
voltage-dependent plant and animal K+ channels share similar pore structures. Through heterologous expression in Xenopus oocytes and voltage-clamp analysis combined with phenotypic analysis involving a ***potassium*** transport-defective Saccharomyces ***cerevisiae*** strain, we investigated the selectivity filter of the mutants and their susceptibility toward ***inhibition*** by cesium and calcium ions. With respect to electrophysiological properties, KAT1 mutants segregated into three groups: (i) wild-type-like channels, (ii) channels modified in selectivity and Cs+ or Ca-2+ sensitivity, and (iii) a group that was additionally affected in its voltage dependence. Despite the additional 14 amino acids in H5, this motif in KAT1 is also involved in the formation of the ion-conducting pore because amino acid substitutions at Leu-251, Thr-256, Thr-259, and Thr-260 resulted in functional channels with modified ionic selectivity and ***inhibition*** . Creation of Ca-2+ sensitivity and an increased susceptibility to Cs+ ***block*** through mutations within the narrow pore might indicate that both ***blockers*** move deeply into the ***channel*** . Furthermore, mutations close to the rim of the pore affecting the half- ***activation*** potential (U-1/2) indicate that amino acids within the pore either interact with the voltage sensor or ion permeation feeds back on gating.

L7 ANSWER 21 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:361768 BIOSIS
DOCUMENT NUMBER: PREV199699084124
TITLE: Use of microphysiometry for analysis of heterologous ion channels expressed in yeast.
AUTHOR(S): Hahnenberger, Karen M.; Krystal, Mark; Esposito, Kim; Tang,
Weimin; Kurtz, Stephen (1)
CORPORATE SOURCE: (1) Biosci. Res. Dev., Hewlett Packard Co., 3500 Deer Creek
Rd., Palo Alto, CA 94304 USA
SOURCE: Nature Biotechnology, (1996) Vol. 14, No. 7, pp. 880-883.
ISSN: 1087-0156.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Measurement of extracellular acidification rates by microphysiometry provides a means to analyze the function of ion channels expressed in yeast cells. These measurements depend on the proton pumping action of the
H+-ATPase, a central component of the yeast plasma membrane. We used microphysiometry to analyze the activity of two ion channels expressed in yeast. In one example, an inwardly rectifying K+ ***channel*** , gpIRK1, provides a ***potassium*** uptake function when expressed in a
potassium transporter-defective yeast strain. Rates of acidification in gpIRK1-expressing cells directly reflect ***channel*** function. Addition of cesium, an ***inhibitor*** of gpIRK1 activity, results in an immediate reduction in acidification rates. In a second example, expression of a nonselective cation ***channel*** , the influenza virus M2 protein, is believed to interfere with the maintenance of the electrochemical proton gradient by the H+-ATPase. In cells expressing the M2 ***channel*** , addition of ***inhibitors*** increases the rate of proton extrusion. Moreover, functional differences between two M2 ***inhibitors*** , amantadine and BL-1743, are distinguished by the microphysiometer. This application demonstrates the utility of the microphysiometer for functional studies of ion channels; it is adaptable to a screening process for compounds that modulate ion ***channel*** activity.

L7 ANSWER 22 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:140708 BIOSIS
DOCUMENT NUMBER: PREV199698712843
TITLE: Identification, expression and evolutionary significance of a member of a novel class of K channels containing two P-regions.
AUTHOR(S): Joiner, W. J. (1); Ketchum, K. A.; Quinn, A. M.; Sellers, A. J.; Goldstein, S. A. N.; Kaczmarek, L. K.
CORPORATE SOURCE: (1) Dep. Cellular Mol. Physiol., Yale Univ. Sch. Med., New
Haven, CT 06520 USA
SOURCE: Biophysical Journal, (1996) Vol. 70, No. 2 PART 2, pp. A152.
Meeting Info.: 40th Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 17-21, 1996
ISSN: 0006-3495.
DOCUMENT TYPE: Conference
LANGUAGE: English

L7 ANSWER 23 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:130307 BIOSIS
DOCUMENT NUMBER: PREV199698702442
TITLE: A pH-sensitive yeast outward rectifier K+ ***channel*** with two pore domains and novel gating properties.
AUTHOR(S): Lesage, Florian; Guillemare, Eric; Fink, Michel; Duprat, Fabrice; Lazdunski, Michel (1); Romey, Georges; Barhanin, Jacques
CORPORATE SOURCE: (1) Inst. de Pharmacol. Mol. Cellulaire, CNRS, 660 Route
des Lucioles, Sophia Antipolis, 06560 Valbonne France
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 8, pp. 4183-4187.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English

AB YORK is a newly cloned K+ ***channel*** from yeast. Unlike all other
cloned K+ channels, it has two pore domains instead of one. It displays eight transmembrane segments arranged like a covalent assembly of a Shaker-type voltage-dependent K+ ***channel*** (without S4 transmembrane segments) with an inward rectifier K+ ***channel*** . When expressed in Xenopus oocytes, YORK does not pass inward currents; it
conducts only K+-selective outward currents. However, the mechanism responsible for this strict outward rectification is unusual. Like inward rectifiers, its ***activation*** potential threshold closely follows the K+ equilibrium potential. Unlike inward rectifiers, the rectification is not due to a voltage-dependent Mg-2+ ***block*** . The blocking element is probably intrinsic to the YORK protein itself. YORK activity is decreased at acidic internal pH, with a pK-a of 6.5. Pharmacological and regulation properties were analyzed. Ba-2+ ions and quinine ***block*** YORK currents through high and low affinity sites, while tetraethylammonium displays only one affinity for blocking. ***Activation*** of protein kinase C indirectly produces an increase of the current, while protein kinase A ***activation*** has no effect.

L7 ANSWER 24 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:539759 BIOSIS
DOCUMENT NUMBER: PREV199598554059
TITLE: YKJC1 encodes the depolarization- ***activated*** K+ ***channel*** in the plasma membrane of yeast.
AUTHOR(S): Zhou, Xin-Liang; Vaillant, Brian; Loukin, Stephen H.; Kung, Ching (1); Saimi, Yoshiro
CORPORATE SOURCE: (1) Lab. Molecular Biol., Univ. Wisconsin, Madison, WI 53706 USA
SOURCE: FEBS Letters, (1995) Vol. 373, No. 2, pp. 170-176. ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Our previous patch-clamp studies showed that depolarization ***activates*** a K+-specific current in the plasma membrane of the budding yeast, Saccharomyces ***cerevisiae*** (Gustin et a). (1986) Science 233, 1195-1197). The Yeast Genome Sequencing Project has now uncovered on the left arm of chromosome X an open reading frame (ORF) that
predicts a 77-kDa protein reminiscent of a shaker-like alpha subunit with 6 membrane spans followed by a subunit with 2 spans. We found that deleting this ORF removes the yeast K+ current. Furnishing the ORF from plasmids restores or even greatly amplifies this current. These manipulations have no effects on the 40-pS mechanosensitive conductance also native to this membrane. Thus, this ORF, named YKC1 here, likely encodes a structure for the K+-specific ***channel*** of the yeast plasma membrane. This and other K+ ***channel*** subunits are compared
and the possible uses of this gene in research are discussed. YKC1 has recently been shown by others to induce in frog oocytes a K+ current. Its ***activation*** is coupled to E-K+ and its outward rectification depends on external divalent cations. We found the YKC1 ***channel***
in its native membrane ***activates*** at low voltages largely independent of E-K+ and it remains so despite removal of divalents by chelation.

L7 ANSWER 25 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:530168 BIOSIS
DOCUMENT NUMBER: PREV199598544468
TITLE: Identification of strong modifications in cation selectivity in an Arabidopsis inward rectifying ***potassium*** ***channel*** by mutant selection in yeast.
AUTHOR(S): Uozumi, Nobuyuki; Gassmann, Walter; Cao, Yongwei; Schroder, Julian I. (1)
CORPORATE SOURCE: (1) Dep. Biol., Cent. Mol. Genetics, Univ. California, San Diego, La Jolla, CA 92093-0116 USA
SOURCE: Journal of Biological Chemistry, (1995) Vol. 270, No. 41, pp. 24276-24281. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The Arabidopsis thaliana cDNA, YAT1, encodes a hyperpolarization- ***activated*** K+ ***channel*** . In the present study, we utilized a combination of random site-directed mutagenesis, genetic screening in a ***potassium*** uptake-deficient yeast strain, and electrophysiological analysis in Xenopus oocytes to identify strong modifications in cation selectivity of the inward rectifying K+ ***channel*** KAT1. Threonine at position 256 was replaced by 11 other amino acid residues. Six of these mutated KAT1 cDNAs complemented a K+ uptake-deficient yeast strain at low
concentrations of ***potassium*** . Among these, two mutants (T256D and
T256G) showed a sensitivity of yeast growth toward high ammonium concentrations and a dramatic increase in current amplitudes of rubidium and ammonium ions relative to K+ by 39-72-fold. These single site mutations gave rise to Rb+- and NH-4+-selective channels with Rb+ and NH-4+ currents that were approximately 10-13-fold greater in amplitude than K+ currents, whereas the NH-4+ to K+ current amplitude ratio of wild
type KAT1 was 0.28. This strong conversion in cation specificity without loss of general selectivity exceeds those reported for other mutations in the pore domain of voltage-dependent K+ channels. Yeast growth was greatly
impaired by sodium in two other mutants at this site (T256E and T256Q), which were blocked by millimolar sodium (K-1/2 = 1.1 mM for T256E), although the wild type ***channel*** was not blocked by 110 mM sodium.
Interestingly, the ability of yeast to grow in the presence of toxic cations correlated to biophysical properties of KAT1 mutants, illustrating the potential for qualitative K+ ***channel*** mutant selection in yeast. These data suggest that the size of the side chain of the amino acid at position 256 in KAT1 is important for enabling cation permeation and that this site plays a crucial role in determining the cation selectivity of hyperpolarization- ***activated*** ***potassium*** channels.

L7 ANSWER 26 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:481298 BIOSIS
DOCUMENT NUMBER: PREV199598495598
TITLE: Functional expression of a vertebrate inwardly rectifying K+ ***channel*** in yeast.
AUTHOR(S): Tang, Weimin; Ruknudin, Abdul; Yang, Wen-Pin; Shaw, Shyh-Yu; Knickerbocker, Aron; Kurtz, Stephen (1)
CORPORATE SOURCE: (1) Bristol-Myers Squibb Pharmaceutical Res. Inst., Princeton, NJ 08543-4000 USA
SOURCE: Molecular Biology of the Cell, (1995) Vol. 6, No. 9, pp. 1231-1240. ISSN: 1059-1524.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We describe the expression of gpIRK1, an inwardly rectifying K+ ***channel*** obtained from guinea pig cardiac cDNA. gpIRK1 is a homologue of the mouse IRK1 ***channel*** identified in macrophage cells. Expression of gpIRK1 in Xenopus oocytes produces inwardly rectifying K+ current, similar to the cardiac inward rectifier current I-K1. This current is blocked by external Ba-2+ and Cs+. Plasmids containing the gpIRK1 coding region under the transcriptional control of constitutive (PGK) or inducible (GAL) promoters were constructed for expression in Saccharomyces ***cerevisiae*** . Several observations suggest that gpIRK1 forms functional ion channels when expressed in yeast.
gpIRK1 complements a trk1-DELTA trk2-DELTA strain, which is defective in
potassium uptake. Expression of gpIRK1 in this mutant restores growth on low ***potassium*** media. Growth dependent on gpIRK1 is
inhibited by external Cs+. The strain expressing gpIRK1 provides a
versatile genetic system for studying the assembly and composition of inwardly rectifying K+ channels.

L7 ANSWER 27 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:432067 BIOSIS

DOCUMENT NUMBER: PREV199598446367
TITLE: A new family of outwardly rectifying ***potassium*** ***channel*** proteins with two pore domains in tandem.
AUTHOR(S): Ketchum, Karen A.; Joiner, William J.; Sellers, Andrew J.;
Kaczmarek, Leonard K.; Goldstein, Steve A. N. (1)
CORPORATE SOURCE: (1) Dep. Cell Mol. Physiol., Boyer Cent. Mol. Med., Yale
Univ. Sch. Med., 295 Congress Ave., New Haven, CT 06536-0812 USA
SOURCE: Nature (London), (1995) Vol. 376, No. 6542, pp. 690-695.
ISSN: 0028-0836.
DOCUMENT TYPE: Article
LANGUAGE: English
AB ***Potassium*** channels catalyse the permeation of K+ ions across cellular membranes and are identified by a common structural motif, a highly conserved signature sequence of eight amino acids in the P domain of each ***channel*** 's pore-forming alpha-subunit. Here we describe a novel K+ ***channel*** (TOK1) from Saccharomyces cevevisiae that contains two P domains within one continuous polypeptide. Xenopus laevis oocytes expressing the ***channel*** exhibit a unique, outwardly rectifying, K+-selective current. The ***channel*** is permeable to outward flow of ions at membrane potentials above the K+ equilibrium potential; its conduction-voltage relationship is thus sensitive to extracellular K+ ion concentration. In excised membrane patches, external divalent cations ***block*** the ***channel*** in a voltage-dependent manner, and their removal in this configuration allows inward ***channel*** current. These attributes are similar to those described for inwardly rectifying K+ channels, but in the opposite direction, a previously unrecognized ***channel*** behaviour. Our results identify a new class of K+ ***channel*** which is distinctive in both its primary structure and functional properties. Structural homologues of the ***channel*** are present in the genome of Caenorhabditis elegans.

L7 ANSWER 28 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:543030 BIOSIS
DOCUMENT NUMBER: PREV199598002578
TITLE: ATP-induced unspecific ***channel*** in yeast mitochondria.
AUTHOR(S): Guerin, Bernard (1); Bunoust, Odile; Rouqueys, Valerie; Rigoulet, Michel
CORPORATE SOURCE: (1) Inst. Biochimie Genetique Cellulaire, Univ. de Bordeaux
2, 1 rue Camille Saint-Saens, 33077 Bordeaux Cedex France
SOURCE: Journal of Biological Chemistry, (1994) Vol. 269, No. 41, pp. 25406-25410.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB ATP induced swelling of isolated yeast mitochondria suspended m an isoomotic solution of ***potassium*** gluconate. Valinomycin stimulated the swelling rate, indicating that K+ influx in the presence of ATP is rate-controlling. This swelling was ***inhibited*** by ADP, phosphate (probably acting on the external face of the inner membrane), and Mg-2+, which forms a complex with ATP. ATP- induced swelling did not require working F-0-F-1-ATPase since it was not ***inhibited*** by oligomycin and uncoupler. CTP and GTP also induced a swelling. ATP also induced mitochondrial swelling in ***potassium*** glutamate, chloride, and acetate but not in phosphate solutions. Sodium, but not ammonium, can replace ***potassium*** ion. It is probable that the ATP-***channel*** opening also necessitates an electrogenic cation influx. Respiration also induced swelling of mitochondria suspended in isoosmotic ***potassium*** gluconate solution. ATP- or respiration-induced swelling were ***inhibited*** equally by N,N'-dicyclohexylcarbodiimide, propranolol, and Zn-2+ but not by quinine; all these drugs ***inhibit*** the H+/K+ exchange. It was concluded that this unspecific ***channel*** is not open under conditions used to measure oxidative phosphorylation. Its physiological role remains unknown.

L7 ANSWER 29 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:405173 BIOSIS
DOCUMENT NUMBER: PREV199497418173
TITLE: Palytoxin induces K+ efflux from yeast cells expressing the mammalian sodium pump.
AUTHOR(S): Scheiner-Bobis, Georgios (1); Heringdorf, Dagmar Meyer Zu;
Christ, Matthias; Habermann, Ernst
CORPORATE SOURCE: (1) Institut fuer Biochemie Endokrinologie, Fachbereich
Veterinaermedizin, Justus-Liebig-Universitaet Giessen, Frankfurter Strasse 100, D-35392 Giessen Germany
SOURCE: Molecular Pharmacology, (1994) Vol. 45, No. 6, pp. 1132-1136.
ISSN: 0026-895X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Palytoxin causes ***potassium*** efflux and sodium influx in all investigated animal cells. Much evidence points to the sodium pump (Na+/K+-ATPase) as the target of the toxin. A heterologous expression system for mammalian Na/K+-ATPase in the brewers yeast Saccharomyces ***cerevisiae*** has been used to test this hypothesis. Yeast cells do not contain endogenous sodium pumps but can be transformed with vectors coding for the alpha and beta subunits of the mammalian sodium pump. We now show that transformed yeast cells expressing both alpha and beta subunits of Na+/K+-ATPase are highly sensitive to the toxin, as measured by the loss of intracellular ***potassium*** . Palytoxin-induced ***potassium*** efflux is completely ***inhibited*** by 500 mu-M ouabain. In contrast, nontransformed yeast cells or cells expressing either the alpha or beta subunits are insensitive to palytoxin. Thus, the alpha/beta heterodimer of the sodium pump is required for the release of ***potassium*** induced by palytoxin. The results suggest that palytoxin converts the sodium pump into an open ***channel*** , allowing the passage of alkali ions.

L7 ANSWER 30 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:279297 BIOSIS
DOCUMENT NUMBER: PREV199396009522
TITLE: Gating and conductance in an outward-rectifying ***potassium*** ***channel*** from the plasma membrane of Saccharomyces ***cerevisiae*** .
AUTHOR(S): Bertl, Adam (1); Slayman, Clifford L.; Gradmann, Dietrich
CORPORATE SOURCE: (1) Pflanzenphysiologisches Institut, Univ. Gottingen,
Untere Karspuele 2, 3400 Gottingen Germany
SOURCE: Journal of Membrane Biology, (1993) Vol. 132, No. 3, pp. 183-199.
ISSN: 0022-2631.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The plasma membrane of the yeast Saccharomyces ***cerevisiae*** has been investigated by patch-clamp techniques, focusing upon the most conspicuous ion ***channel*** in that membrane, a K+-selective ***channel*** . In simple observations on inside-out patches, the ***channel*** is predominantly closed at negative membrane voltages, but opens upon polarization towards positive voltages, typically displaying long flickery openings of several hundred milliseconds, separated by long gaps (G). Elevating cytoplasmic calcium shortens the gaps but also introduces brief ***blocks*** (B, closures of 2-3 msec duration). On the assumption that the flickery open intervals constitute bursts of very brief openings and closings, below the time resolution of the recording system, analysis via the beta distribution revealed typical closed durations (interrupts, I) near 0.3 msec, and similar open durations. Overall behavior of the ***channel*** is most simply described by a kinetic model with a single open state (O), and three parallel closed states with significantly different lifetimes: long (G), short (B) and very short (I). Detailed kinetic analysis of the three open/closed

transitions, particularly with varied membrane voltage and cytoplasmic calcium concentration, yielded the following stability constants for ***channel*** closure: K-I = 3.3 cntdot e-z in which u = eV-m/kT is the reduced membrane voltage, and z is the charge number; K-G = 1.9 cntdot 10-4((Ca-2+) cntdot e-zu)-1; and K-B = 2.7 cntdot 10-3((Ca-2+) cntdot e-zu)-2. Because of the ***antagonistic*** effects of both membrane voltage (V-m) and cytoplasmic calcium concentration ((Ca-2+)-cyt) on ***channel*** opening from the B state, compared with openings from the G state, plots of net open probability (P-o) us. either V-m or (Ca-2+) are bell-shaped, approaching unity at low calcium (mu-M) and high voltage (+150 mV), and approaching 0.25 at high calcium (10 mM) and zero voltage.

Current-voltage curves of the open ***channel*** are sigmoid vs. membrane voltage, saturating at large positive or large negative voltages; but time-averaged currents, along the rising limb of P-o (in the range 0 to +150 mV, for 10 mu-M Ca-2+)) make this ***channel*** a strong outward rectifier. The overall properties of the ***channel*** suggest that it functions in balancing charge movements during secondary active transport in Saccharomyces.

L7 ANSWER 31 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1992:304005 BIOSIS
DOCUMENT NUMBER: BA94:17155
TITLE: FUNCTIONAL EXPRESSION OF A PROBABLE ARABIDOPSIS-THALIANA ***POTASSIUM*** ***CHANNEL*** IN SACCHAROMYCES-***CEREVISIAE*** .
AUTHOR(S): ANDERSON J A; HUPRIKAR S S; KOCHIAN L V; LUCAS W J; GABER R F
CORPORATE SOURCE: DEP. BIOCHEM., MOL. BIOL., NORTHWESTERN UNIV., EVANSTON, ILL. 60208.
SOURCE: PROC NATL ACAD SCI U S A, (1992) 89 (9), 3736-3740.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB We report the isolation of a cDNA (KAT1) from Arabidopsis thaliana that encodes a probable K+ ***channel*** . KAT1 was cloned by its ability to suppress a K+ transport-defective phenotype in mutant Saccharomyces ***cerevisiae*** cells. This suppression is sensitive to known K+ ***channel*** ***blockers*** , including tetraethylammonium and Ba2+ ions. The KAT1 cDNA contains an open reading frame capable of encoding a 78-kDa protein that shares structural features found in the Shaker superfamily of K+ channels. These include a cluster of six putative membrane-spanning helices (S1-S6) at the amino terminus of the protein, a presumed voltage-sensing region containing Arg/Lys-Xaa-Xaa-Arg/Lys repeats within S4, and the highly conserved pore-forming region (known as H5 or SS1-SS2). Our results suggest that the structural motif for K+ channels has been conserved between plants and animals.

L7 ANSWER 32 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1991:68103 BIOSIS
DOCUMENT NUMBER: BA91:36763
TITLE: REGULATION OF ***POTASSIUM*** FLUXES IN SACCHAROMYCES-***CEREVISIAE*** .
AUTHOR(S): RAMOS J; HARO R; RODRIGUEZ-NAVARRO A
CORPORATE SOURCE: DEP. MICROBIOL., ESCUELA TECNICA SUPERIOR DE INGENIEROS AGRONOMOS, E-28040 MADRID, SPAIN.
SOURCE: BIOCHIM BIOPHYS ACTA, (1990) 1029 (2), 211-217.
CODEN: BBACAQ. ISSN: 0006-3002.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB To investigate the regulation of K+ fluxes in Saccharomyces ***cerevisiae*** the dependence of K+ efflux and Rb+ influx on [K+]i, [Na+]i, membrane potential, cell volume, and turgor pressure were studied in cells with different K+ contents. By decreasing the cell volume with osmotic shocks and the cellular pH with butyric acid the following was found. (1) The K+ efflux induced by uncouplers decreases simultaneously with the decrease of the K+ content of the cell, but the process was insensitive to [K+]i, pHi, cell volume and turgor pressure. The internal presence of Na+ ***inhibited*** this K+ efflux. (2) The increase of the Vmax of Rb+ influx observed in low-K+ cells is due to the decrease of the pHi and probably mediated by the increase of the activity of the plasma membrane ATPase. The Vmax is independent of [K+]i, [Na+]i, cell volume and turgor pressure. (3) The decrease in the Km of Rb+ influx observed in low-K+ cells does not depend directly on [K+]i, pHi, cell volume or turgor pressure. If Na+ is present, [Na+]i might be directly involved in the regulation of the Km.

L7 ANSWER 33 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1990:445972 BIOSIS
DOCUMENT NUMBER: BA90:96612
TITLE: A PUTATIVE ***POTASSIUM*** -ION SELECTIVE ***CHANNEL*** IN THE PLASMA MEMBRANE OF YEAST THAT IS BLOCKED BY MICROMOLAR CONCENTRATIONS OF EXTERNAL DIVALENT CATIONS AND IS INSENSITIVE TO TETRAETHYLAMMONIUM.
AUTHOR(S): VAN DE MORTEL J B J; THEUVENET A P R; BORST-PAUWELS G W F H
CORPORATE SOURCE: LAB. CELL BIOL., R. C. UNIV., FAC. SCI., TOERNOOIVELD, 6525 ED NIJMEGEN, THE NETHERLANDS.
SOURCE: BIOCHIM BIOPHYS ACTA, (1990) 1026 (2), 220-224.
CODEN: BBACAQ. ISSN: 0006-3002.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB At pH 7, addition of glucose to an anaerobic suspension of non-metabolizing yeast cells [Saccharomyces ***cerevisiae***], causes a transient net efflux of K+ from the cells and a concomitant transient hyperpolarization of the plasma membrane (Van de Mortel, J.B.J., et al. (1988) Biochim. Biophys. Acta 936, 421-428). Both phenomena are effectively suppressed in the presence of low concentrations of polyvalent cations. The concentrations of Mn2+, Ca2+, Ba2+, Mg2+, Sr2+ and La3+ required for half-maximal suppression of the transient hyperpolarization are 10, 17, 20, 38, 47 and 5 .mu.M, respectively. Subsequent addition of EDTA 90 s after that of Ca2+ immediately restores both K+ efflux and cellular uptake of the fluorescent membrane potential probe 2-(dimethylaminostyryl)1-1ethylpyridinium (DMP). This suggests that an interaction of polyvalent cations with an external binding site ***blocks*** the putative K+-selective ***channel*** . Opening of this ***channel*** is not blocked by 20 mM tetraethylammonium nor by 100 .mu.M 3,4-diaminopyridine. It is argued that this glucose-induced K+-conductive pathway is not identical to the voltage-gated K+ channels identified until now in patch-clamp studies of the yeast plasma membrane.

L7 ANSWER 34 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1990:153224 BIOSIS
DOCUMENT NUMBER: BA89:80642
TITLE: FURTHER CHARACTERIZATION OF THE CATION ***CHANNEL*** OF A YEAST VACUOLAR MEMBRANE IN A PLANAR LIPID BILAYER.
AUTHOR(S): SATO M; TANIFUJI M; KASAI M
CORPORATE SOURCE: DEP. BIOPHYS. ENG., FAC. ENG. SCI., OSAKA UNIV., TOYONAKA, OSAKA 560, JAPAN.
SOURCE: CELL STRUCT FUNCT, (1989) 14 (6), 659-668.
CODEN: CSFUDY. ISSN: 0386-7196.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A voltage-dependent and Ca2+- ***activated*** cation ***channel*** recently found in the vacuolar membrane of the yeast Saccharomyces ***cerevisiae*** was incorporated into planar lipid bilayers and further characterized in macroscopic and single ***channel*** levels. Single ***channel*** conductances for various cations were in the order:

NH4+ >
K+ > Rb+ > Cs+ > Na+ > Li+, and were nearly consistent with the order of
permeability ratio estimated from reversal potentials determined by macroscopic measurement. Up to 6 mM of Ca2+ added to the cis (cytoplasmic) side opened the ***channel*** , but higher concentrations closed the ***channel*** without affecting the single ***channel*** conductance. Ba2+ closed the ***channel*** without opening. Further, large anions such as gluconate closed the ***channel*** from the cis side. In addition to the above ***channel*** , a small cation-selective ***channel*** of about 40 pS was found.

L7 ANSWER 35 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1990:4050 BIOSIS
DOCUMENT NUMBER: BA89:4050
TITLE: ATP-SENSITIVE ***POTASSIUM*** CHANNELS IN A PLASMA
MEMBRANE PROTON ATPASE MUTANT OF THE YEAST SACCHAROMYCES-
CEREVISIAE .
AUTHOR(S): RAMIREZ J A; VACATA V; MCCUSKER J H; HARBER J E; MORTIMER R
K; OWEN W G; LECAR H
CORPORATE SOURCE: DEP. BIOPHYSICS AND LAWRENCE BERKELEY LAB., UNIV.
CALIFORNIA, BERKELEY, CALIF. 94720.
SOURCE: PROC NATL ACAD SCI U S A, (1989) 86 (20), 7866-7870.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A mutant in the plasma membrane H+-ATPase gene of the yeast Saccharomyces
cerevisiae with a reduced H+-ATPase activity, when examined at the
single- ***channel*** level with the patch-clamp technique, was found to exhibit K+ channels ***activated*** by intracellular application of ATP. In the parent strain, the same ***channel*** , identified by its conductance and selectivity, is not ***activated*** by ATP. This activity in the mutant is blocked by the ATPase ***inhibitor*** N,N'-dicyclohexylcarbodiimide. ADP and the ATP analog adenosine 5'-[.gamma.-[35S]thio]triphosphate do not ***activate*** the ***channel*** . These findings suggest a tight physical coupling between
the plasma membrane ATPase and the K+ ***channel*** .

L7 ANSWER 36 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1989:215468 BIOSIS
DOCUMENT NUMBER: BR36:104682
TITLE: YEAST ION CHANNELS SELECTIVITY AND ***INHIBITION*** OF
NUTRIENT UPTAKE BY ***POTASSIUM*** ***CHANNEL***
BLOCKERS .
AUTHOR(S): GOMEZ-LAGUNAS F; PENA A; DARSZON A
CORPORATE SOURCE: DEP. BIOENERGETICS, IFC-UNAM, APDO. POSTAL 04510, MEXICO
CITY.
SOURCE: THIRTY-THIRD ANNUAL MEETING OF THE BIOPHYSICAL SOCIETY,
CINCINNATI, OHIO, USA, FEBRUARY 12-16, 1989. BIOPHYS J,
(1989) 55 (2 PART 2), 544A.
CODEN: BIOJAU. ISSN: 0006-3495.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L7 ANSWER 37 OF 55 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1986:440031 BIOSIS
DOCUMENT NUMBER: BA82:106219
TITLE: IONS CHANNELS IN YEAST SACCHAROMYCES-***CEREVISIAE*** .
AUTHOR(S): GUSTIN M C; MARTINAC B; SAIMI Y; CULBERTSON M R; KUNG C
CORPORATE SOURCE: LAB. MOLECULAR BIOLOGY, UNIV. WISCONSIN-MADISON, MADISON,
WI 53706.
SOURCE: SCIENCE (WASH D C), (1986) 233 (4769), 1195-1197.
CODEN: SCIEAS. ISSN: 0036-8075.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Voltage-dependent ion channels have been found in the plasma membrane of
the yeast Saccharomyces ***cerevisiae*** . Ion ***channel*** activities were recorded from spheroplasts or patches of plasma membrane with the patch-clamp technique. The most prominent activities came from a
set of ***potassium*** channels with the properties of ***activation*** by positive but not negative voltages, high selectivity for ***potassium*** over sodium ion, unit conductance of 20 picosiemens, ***inhibition*** by tetraethylammonium or barium ions, and bursting kinetics.

L7 ANSWER 38 OF 55 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999114557 EMBASE
TITLE: ***Channel*** -mediated high-affinity K+ uptake into guard cells from Arabidopsis.
AUTHOR: Bruggemann L.; Dietrich P.; Becker D.; Dreyer I.; Palme K.;
Hedrich R.
CORPORATE SOURCE: R. Hedrich, J.-von-Sachs-Inst. Biowissenschaften, Lehrs.
Molek. Pflanzenphys./Biophys., Universitat Wurzburg, Julius-von-Sachs-Platz 2, 97082 Wurzburg, Germany
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1999) 96/6 (3298-3302).
Refs: 35
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB ***Potassium*** uptake by higher plants is the result of high- or low-affinity transport accomplished by different sets of transporters.
Although K+ channels were thought to mediate low-affinity uptake only, the
molecular mechanism of the high-affinity, proton-dependent K+ uptake system is still scant. Taking advantage of the high-current resolution of the patch-clamp technique when applied to the small Arabidopsis thaliana guard cells densely packed with voltage-dependent K+ channels, we could directly record channels working in the concentration range of high-affinity K+ uptake systems. Here we show that the K+ ***channel***
KAT1 expressed in Arabidopsis guard cells and yeast is capable of mediating ***potassium*** uptake from media containing as little as 10 .mu.M of external K+. Upon reduction of the external K+ content to the micromolar level the voltage dependence of the ***channel*** remained
unaffected, indicating that this ***channel*** type represents a voltage sensor rather than a K+-sensing valve. This behavior results in K+ release through K+ uptake channels whenever the Nernst potential is negative to the ***activation*** threshold of the ***channel*** .
In contrast to the H+coupled K+ symport shown to account for high-affinity
K+ uptake in roots, pH-dependent K+ uptake into guard cells is a result of a shift in the voltage dependence of the K+ ***channel*** . We conclude
that plant K+ channels ***activated*** by acid pH may play an essential role in K+ uptake even from dilute solutions.

L7 ANSWER 39 OF 55 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998141183 EMBASE
TITLE: TOK1 is a volatile anesthetic stimulated K+ ***channel***
AUTHOR: Gray A.T.; Winegar B.D.; Leonoudakis D.J.; Forsayeth J.R.;
Yost C.S.
CORPORATE SOURCE: Dr. C.S. Yost, Anesthesia Research Laboratory, Univ. of

California Medical Center, 513 Parnassus Avenue, San Francisco, CA 94143-0542, United States
SOURCE: Anesthesiology, (1998) 88/4 (1076-1084).
Refs: 41
ISSN: 0003-3022 CODEN: ANESAV
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 024 Anesthesiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Background: Volatile anesthetic agents can ***activate*** the S ***channel*** , a baseline ***potassium*** (K+) ***channel*** , of the marine mollusk Aplysia. To investigate whether cloned ion channels (***potassium*** selectivity, outward rectification, and ***activation*** independent of voltage) also are modulated by volatile anesthetic agents, the authors expressed the cloned yeast ion ***channel*** TOK1 (tandem pore domain, outwardly rectifying K+ ***channel***) in Xenopus oocytes and studied its sensitivity to volatile agents. Methods: Standard two-electrode voltage and patch clamp recording methods were used to study TOK1 channels expressed in Xenopus oocytes. Results: Studies with two- electrode voltage clamp at room temperature showed that halothane, isoflurane, and desflurane increased TOK1 outward currents by 48-65% in barium Frog Ringer's perfusate. The concentrations at which 50% potentiation occurred (EC50 values) were in the range of 768-814 .mu.M (0.016-0.044 atm) and had a rank order of potency in atm in which halothane > isoflurane > desflurane. The potentiation of TOK1 by volatile anesthetic agents was rapid and reversible (onset and offset, 1-20 s). In contrast, the nonanesthetic 1,2-dichlorohexafluorocyclobutane did not potentiate TOK1 currents in concentrations up to five times the MAC value predicted by the Meyer-Overton hypothesis based on oil/gas partition coefficients. Single TOK1 ***channel*** currents were recorded from excised outside-out patches. The single ***channel*** open probability increased as much as twofold in the presence of isoflurane and rapidly returned to the baseline values on washout. Volatile anesthetic agents did not alter the TOK1 single ***channel*** current-voltage (I-V) relationship, however, suggesting that the site of action does not affect the permeation pathway of the ***channel*** . Conclusion: TOK1 is a ***potassium*** ***channel*** that is stimulated by volatile anesthetic agents. The concentrations over which potentiation occurred (EC50 values) were higher than those commonly used in clinical practice (approximately twice MAC).

L7 ANSWER 40 OF 55 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96146489 EMBASE
DOCUMENT NUMBER: 1996146489
TITLE: Response of Saccharomyces ***cerevisiae*** to changes in external osmolarity.
AUTHOR: Varela J.C.S.; Mager W.H.
CORPORATE SOURCE: Dept. Biochemistry Molecular Biology, Inst. Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Univ., De Boelelaan 1083,1081 HV Amsterdam, Netherlands
SOURCE: Microbiology, (1996) 142/4 (721-731).
ISSN: 1350-0872 CODEN: MROBEO
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
LANGUAGE: English

L7 ANSWER 41 OF 55 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96038472 EMBASE
DOCUMENT NUMBER: 1996038472
TITLE: Palytoxin-induced Na+ influx into yeast cells expressing the mammalian sodium pump is due to the formation of a ***channel*** within the enzyme.
AUTHOR: Redondo J.; Fiedler B.; Scheiner-Bobis G.
CORPORATE SOURCE: Inst. fur Biochemie/Endokrinologie, Fachbereich Veterinarmedizin, Justus-Liebig-Universitat Giessen, Frankfurter Str. 100,D-35392 Giessen, Germany
SOURCE: Molecular Pharmacology, (1996) 49/1 (49-57).
ISSN: 0026-895X CODEN: MOPMA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 052 Toxicology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Palytoxin forms ionic channels in animal cell membranes but does not have similar effects on bacteria or yeast cells. These channels appear to be associated with the sodium pump. Using a heterologous expression system for the mammalian sodium pump in the yeast Saccharomyces ***cerevisiae*** , we recently demonstrated palytoxin-induced K+ efflux from yeast cells. Using the same system, we now show that the palytoxin-induced Na+ influx measured by others in animal cells is also directly associated with the sodium pump. Under the influence of palytoxin, yeast cells that express the mammalian sodium pump exchange extracellular Na+ ions for intracellular K+ ions with a stoichiometry of .apprx.1:1. Both fluxes can be ***inhibited*** by ouabain. K+ efflux can also be observed when extracellular Na+ is replaced by Li+, Cs+, or NH4+. These data suggest that all palytoxin-induced ion fluxes measured so far in various cell systems are directly associated with the sodium pump. Palytoxin-induced Na+ influx or K+ efflux does not occur with yeast cells that express a truncated form of the sodium pump that is missing 44 of the carboxyl-terminal amino acids of the .alpha.1 subunit. Scatchard analysis reveals only a slightly lower affinity of the truncated form for [3H]ouabain compared with the affinity of the native enzyme. Yeast cells expressing the truncated enzyme can bind [3H]ouabain, which can be displaced by palytoxin. Therefore, the inability of the truncated form to conduct ions under the influence of palytoxin is not due to the removal of the palytoxin binding site but rather to the removal of a part of the enzyme that participates in a direct or indirect way in the formation of the palytoxin-induced ***channel*** . Based on these findings, we conclude that palytoxin opens a ***channel*** within and not merely in the vicinity of the sodium pump. This might be the same ***channel*** that under normal conditions actively transports Na+ and K+ ions.

L7 ANSWER 42 OF 55 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95253389 EMBASE
DOCUMENT NUMBER: 1995253389
TITLE: An electrophysiological study of yeast mitochondria. Evidence for two inner membrane anion channels sensitive to ATP.
AUTHOR: Ballarin C.; Sorgato M.C.
CORPORATE SOURCE: Dipartimento di Chimica Biologica, Universita di Padova, Via Trieste 75,35121 Padova, Italy
SOURCE: Journal of Biological Chemistry, (1995) 270/33 (19262-19268).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The inner membrane of mitochondria from various strains of Saccharomyces ***cerevisiae*** has been analyzed with the patch clamp technique for comparison with the better known homologous membrane in mammals (Sorgato, M. C., and Moran, O. (1993) CRC Crit. Rev. Biochem. Mol. Biol. 18, 127-171). Differently than in mammals, the yeast inner membrane was found to harbor essentially two channels with similar anionic selectivity but otherwise different functional behavior. One had a conductance of around 45 picosiemens (in symmetrical 150 mM KCl) and an activity only marginally sensitive to voltage. The other ***channel*** was prominent for the higher outwardly rectifying current and for the dependence upon voltage of the open probability that induced rapid closure at physiological (negative) membrane potentials. Particularly interesting was the effect of ATP (Mg2+ free) added on the matrix side of the membrane. In the case of the lower conducting ***channel*** , the nucleotide caused an

immediate
block of activity (IC50, 0.240 mM), whereas it locked the larger conductance in the open state at both positive and negative potentials. In proteoliposomes containing both mitochondrial membranes, the small conductance was clearly evident, whereas a larger ***channel*** , cationic and without the voltage dependence typical of that in the native inner membrane, was found.

L7 ANSWER 43 OF 55 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95163834 EMBASE
DOCUMENT NUMBER: 1995163834
TITLE: In vivo stimulation of H+-ATPase in various yeast species by diacylglycerols.
AUTHOR: Kotyk A.; Georghiou G.
CORPORATE SOURCE: Department of Membrane Transport, Institute of Physiology,
Czech Academy of Science,142 20 Prague, Czech Republic
SOURCE: Bulletin of Molecular Biology and Medicine, (1994) 19/2-4
(127-133).
ISSN: 0391-481X CODEN: BMBMD5
COUNTRY: Italy
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Dioctanoylglycerol (DOcG), an ***activator*** of protein kinase C which, in its turn, causes the plasma membrane H+-ATPase of yeast to extrude protons, accomplishes the ***activation*** even in vivo in cells of Saccharomyces ***cerevisiae*** , Lodderomyces elongisporus, Rhodotorula gracilis, Dipodascus magnusii and Schizosaccharomyces pombe.
ATP hydrolysis by the ATPase in purified membranes was not sensitive to the presence of DOcG, apparently because of the absence of protein kinase
C from the preparations. The DOcG-induced acidification was not influenced
by the presence of K+ and Tl+ which greatly enhanced the glucose-induced
acidification, this supports the view of separate exchange channels or carriers for H+ vs. K+ and the like. Like glucose, DOcG increased the initial rate of uptake of H+-symported adenine and glutamic acid, attesting to the direct involvement of protons provided by the plasma membrane H+-ATPase in these symports.

L7 ANSWER 44 OF 55 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92007059 EMBASE
DOCUMENT NUMBER: 1992007059
TITLE: Transport of lactic acid in Kluyveromyces marxianus: Evidence for a monocarboxylate uniport.
AUTHOR: Fonseca A.; Spencer-Martins I.; Van Uden N.
CORPORATE SOURCE: Laboratory of Microbiology, Gulbenkian Institute of
Science, 2781 Oeiras Codex, Portugal
SOURCE: Yeast, (1991) 7/8 (775-780).
ISSN: 0749-503X CODEN: YESTE3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Lactic acid-grown cells of a strain of Kluyveromyces marxianus transported
D- and L-lactic acid by a saturable mechanism that was partially inducible and subject to glucose repression, with the following kinetic parameters at pH 5.4: V(max) = 1.00 (.+-. 0.13) mmol h-1 per g dry weight and K(s) =
0.42 (.+-. 0.08) mM. Lactic acid transport was competitively ***inhibited*** by pyruvic, glycolic, acetic and bromoacetic acids. The latter, a non-metabolizable analogue, was transiently accumulated, the extent depending on the extracellular pH. The pH dependence of the K(s) values for undissociated lactic acid and for the lactate anion indicated that the latter was the transported species. Lactate uptake was not accompanied by the simultaneous uptake of protons, ***potassium*** ions or sodium ions excluding symport mechanisms. Initial lactic acid uptake led to transient membrane hyperpolarization as measured with a fluorescent dey excluding also an electroneutral anion antiport mechanism. It was concluded that lactate anions use a monocarboxylate uniport and that the counter anion, possibly bicarbonate, uses a separate ***channel*** , the coupling being electrical and loose.

L7 ANSWER 45 OF 55 MEDLINE
ACCESSION NUMBER: 2000084092 MEDLINE
DOCUMENT NUMBER: 20084092 PubMed ID: 10616719
TITLE: Phage abortive infection of Bacillus licheniformis ATCC 9800; identification of the abiBL11 gene and localisation and sequencing of its promoter region.
AUTHOR: Tran L S; Szabo L; Ponyi T; Orosz L; Sik T; Holczinger A
CORPORATE SOURCE: Department of Biotechnology and Molecular Genetics,
University of Agricultural Sciences, Godollo, Hungary.
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, ***(1999 Nov)***
52 (6) 845-52.
Journal code: AMC; 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF062650
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000214
AB The virulent bacteriophage BL11 infects almost all Bacillus licheniformis
strains tested, including the industrial bacitracin-producing B. licheniformis 19. B. licheniformis ATCC 9800, however, was virtually insensitive to phage BL11 infection, and all of the few surviving progeny phages proved to be mutants. The phage-resistance mechanism was neither
inhibition of adsorption, nor restriction or exclusion provided by a resident prophage, but was, instead, of another type. Phage BL11 adsorbed well on to ATCC 9800 cells, its DNA was injected, but replication
of phage DNA was ***inhibited*** and the infected cells died. Thus, the mechanism of phage resistance was identified as abortive infection (AbiBL11). The so-called abiBL11 gene was identified on the chromosome of
strain ATCC 9800 by Tn917PF1 transposon mutagenesis. Part of the abiBL11
gene from the phage-sensitive ATCC 9800::Tn917PFI was cloned. Gene-disruption analysis, based on Campbell-type integration, showed that
a 0.3-kb EcoRI fragment contained the 5' end of abiBL11. The promoter region of abiBL11 was identified using promoter- and terminator-probe plasmids. The deduced sequence (206 amino acids) of the N-terminal part of
abiBL11 showed no significant homology to known abortive-infection genes,
but did show homology to a Saccharomyces ***cerevisiae*** gene coding
for a serine/threonine protein kinase (RCK1).

L7 ANSWER 46 OF 55 MEDLINE
ACCESSION NUMBER: 1999084969 MEDLINE
DOCUMENT NUMBER: 99084969 PubMed ID: 9864342
TITLE: Divalent cation ***block*** of inward currents and low-affinity K+ uptake in Saccharomyces ***cerevisiae***
AUTHOR: Roberts S K; Fischer M; Dixon G K; Sanders D
CORPORATE SOURCE: Plant Laboratory, Department of Biology, University of
York, York YO1 5YW, United. Kingdom.skr4@york.ac.uk
SOURCE: JOURNAL OF BACTERIOLOGY, ***(1999 Jan)*** 181 (1)
291-7.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990223
Last Updated on STN: 19990223
Entered Medline: 19990205
AB We have used the patch clamp technique to characterize whole-cell currents
in spheroplasts isolated from a trk1Delta trk2Delta strain of Saccharomyces ***cerevisiae*** which lacks high- and moderate-affinity
K+ uptake capacity. In solutions in which extracellular divalent cation concentrations were 0.1 mM, cells exhibited a large inward current. This current was not the result of increasing leak between the glass pipette and membrane, as there was no effect on the outward current. The inward current comprised both instantaneous and time-dependent components. The
magnitude of the inward current increased with increasing extracellular K+ and negative membrane potential but was insensitive to extracellular anions. Replacing extracellular K+ with Rb+, Cs+, or Na+ only slightly modulated the magnitude of the inward current, whereas replacement with Li+ reduced the inward current by approximately 50%, and tetraethylammonium (TEA+) and choline were relatively impermeant. The inward current was blocked by extracellular Ca2+ and Mg2+ with apparent
Kis (at -140 mV) of 363 +/- 78 and 96 +/- 14 microM, respectively. Furthermore, decreasing cytosolic K+ increased the magnitude of the inward
current independently of the electrochemical driving force for K+ influx, consistent with regulation of the inward current by cytosolic K+. Uptake of 86Rb+ by intact trk1Delta trk2Delta cells was ***inhibited*** by extracellular Ca2+ with a Ki within the range observed for the inward current. Furthermore, increasing extracellular Ca2+ from 0.1 to 20 mM significantly ***inhibited*** the growth of these cells. These results are consistent with those of the patch clamp experiments in suggesting that low-affinity uptake of alkali cations in yeast is mediated by a transport system sensitive to divalent cations.

L7 ANSWER 47 OF 55 MEDLINE
ACCESSION NUMBER: 96303826 MEDLINE
DOCUMENT NUMBER: 96303826 PubMed ID: 8723646
TITLE: The S. ***cerevisiae*** outwardly-rectifying ***potassium*** ***channel*** (DUK1) identifies a new family of channels with duplicated pore domains.
AUTHOR: Reid J D; Lukas W; Shafaatian R; Bertl A; Scheurmann-Kettner C; Guy H R; North R A
CORPORATE SOURCE: Glaxo Institute for Molecular Biology, Geneva, Switzerland.
SOURCE: RECEPTORS AND CHANNELS, ***(1996)*** 4 (1) 51-62.
Journal code: B3Y; 9315376. ISSN: 1060-6823.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X94403
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19990129
Entered Medline: 19961107
AB ***Potassium*** ***channel*** subunits have six or two transmembrane segments in addition to a conserved pore-forming (P) domain;
four subunits come together to form a ***channel*** . A gene was identified in S. ***cerevisiae*** (J0911) encoding a protein with eight probable membrane-spanning segments and two such P regions. This protein (Duk1p) is a ***potassium*** ***channel*** because Xenopus
oocytes injected with the corresponding RNA express ***potassium*** currents ***activated*** by depolarization that are not seen in control oocytes. Similar ***potassium*** currents were recorded from wildtype S. ***cerevisiae*** spheroplasts, but not from those in which the DUK1 locus had been disrupted. Cells carrying the duk1 delta 1::HIS disruption in addition to a chimeric gene comprising DUK1 behind the GAL1
promoter showed outward currents when grown in galactose, but not when grown in glucose. Additional sequences with the duplicate pore motif were found in C. elegans, suggesting that these proteins represent a novel structural family of ***potassium*** ***channel*** proteins.

L7 ANSWER 48 OF 55 MEDLINE
ACCESSION NUMBER: 96163193 MEDLINE
DOCUMENT NUMBER: 96163193 PubMed ID: 8550001
TITLE: Inward and outward rectifying ***potassium*** currents in Saccharomyces ***cerevisiae*** mediated by endogenous and heterelogously expressed ion channels.
AUTHOR: Bertl A; Anderson J A; Slayman C L; Sentenac H; Gaber R F
CORPORATE SOURCE: Department of Cellular and Molecular Physiology, Yale
University, New Haven, CT 06510, USA.
SOURCE: FOLIA MICROBIOLOGICA, ***(1994)*** 39 (6) 507-9.
Journal code: F23; 0376757. ISSN: 0015-5632.
PUB. COUNTRY: Czech Republic
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 19960306
Last Updated on STN: 19990129
Entered Medline: 19960220
AB Disruption of genes encoding endogenous transport proteins in Saccharomyces ***cerevisiae*** has facilitated the recent cloning, by functional expression, of cDNAs encoding K+ channels and amino acid transporters from the plant Arabidopsis thaliana [1-4]. In the present study, we demonstrate in whole-cell patch clamp experiments that the inability of trk1deltatrk2delta mutants of S. ***cerevisiae*** to grow on submillimolar K+ correlates with the lack of K+ inward currents, which are present in wild-type cells, and that transformation of the trk1deltatrk2delta double-deletion mutant with KAT1 from Arabidopsis thaliana restores this phenotype by encoding a plasma membrane protein that allows large K+ inward currents. Similar K+ inward currents are induced by transformation of a trk1 mutant with AKT1 from A. thaliana.

L7 ANSWER 49 OF 55 MEDLINE
ACCESSION NUMBER: 89141739 MEDLINE
DOCUMENT NUMBER: 89141739 PubMed ID: 2465412
TITLE: Gating behaviors of a voltage-dependent and Ca2+-***activated*** cation ***channel*** of yeast vacuolar membrane incorporated into planar lipid bilayer.
AUTHOR: Tanifuji M; Sato M; Wada Y; Anraku Y; Kasai M
CORPORATE SOURCE: Department of Biophysical Engineering, Faculty of
Engineering Science, Osaka University, Japan.
SOURCE: JOURNAL OF MEMBRANE BIOLOGY, ***(1988 Nov)*** 106 (1)
47-55.
Journal code: J4E; 0211301. ISSN: 0022-2631.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198903
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19960129
Entered Medline: 19890329
AB A voltage-dependent and Ca2+- ***activated*** cation ***channel***
found in the vacuolar membrane of the yeast, Saccharomyces ***cerevisiae*** , was incorporated into planar lipid bilayer and its gating characteristics were studied at the macroscopic and single-***channel*** levels. The open- ***channel*** probability at steady -state, which was estimated by the macroscopic current measurement, gave a
maximum value at -10 mV and decreased in a graded fashion as the voltage
became more positive or more negative. The steady-state voltage dependence
was explained by assuming two independent gates, which had different rate
constants and opposite voltage dependence. The fast-responding gate opened
when the voltage of the cis side (the side to which the vesicles were added) was made more negative and the slow-responding gate behaved in the
opposite direction. Relatively high concentrations of Ca2+, about 1 mM, were required on the cis side for opening the slow gate in a

voltage-dependent manner. DIDS increased the open- ***channel*** probability of the fast gate when added to the cis side, but was ineffective on the slow gate.

L7 ANSWER 50 OF 55 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1993-368781 [46] WPIDS
DOC. NO. CPI: C1993-163717
TITLE: New DNA encoding plant ***potassium*** ***channel*** - e.g. in yeast strains, for identifying ***potassium*** ***channel*** ***inhibitors*** , potential herbicides and drugs.
DERWENT CLASS: C06 D16
INVENTOR(S): GABER, R F
PATENT ASSIGNEE(S): (NOUN) UNIV NORTHWESTERN
COUNTRY COUNT: 19
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9322422	A1	19931111	(199346)*	EN	39 <--
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE					
W: CA JP					
US 5795770	A	19980818	(199840)		<--

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9322422	A1	WO 1993-US3942	19930421
US 5795770	A CIP of	US 1992-874846	19920427
	Cont of	US 1992-923094	19920731
		US 1997-795788	19970205

PRIORITY APPLN. INFO: US 1992-923094 19920731; US 1992-874846 19920427; US 1997-795788 19970205
AN 1993-368781 [46] WPIDS
AB WO 9322422 A UPAB: 19940103
cDNA (I) encoding a plant ***potassium*** ***channel*** is new.
Also new are (1) genetically engineered strains of yeast, contg. a heterologous ion ***channel*** , consisting of a K- ***channel*** defective phenotypic yeast strain transformed with DNA that suppresses the phenotype.
Pref. the yeast is Saccharomyces ***cerevisiae*** in which the genes TRK1 and opt. also TRK2 are deleted or mutated, and the added DNA is the specified 2173 bp sequence.
USE/ADVANTAGE - The new yeasts are used to detect expression of heterologous ion channels, i.e. to screen for new herbicides and drugs (***inhibitors*** of K channels), potentially reducing costs and time, since the cells are easily adapted for microtitre plate methods, with growth/ ***inhibition*** detected by turbidity.
Dwg.0/4

L7 ANSWER 51 OF 55 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:471456 HCAPLUS
DOCUMENT NUMBER: 129:93574
TITLE: Cloning and expression of a cDNA for the .beta. subunit of a mammalian calcium- ***activated*** ***potassium*** ***channel*** and its use in the screening for modulators of ***channel*** activity
INVENTOR(S): Kaczorowski, Gregory J.; Garcia, Maria L.; Leonard, Reid J.; McManus, Owen B.; Swanson, Richard J.; Folander, Kimberly L.
PATENT ASSIGNEE(S): Merck and Co., Inc., USA
SOURCE: U.S., 22 pp. Cont.-in-part of U. S. 5,637,470.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5776734	A	19980707	US 1996-732506	19961112 <--
US 5637470	A	19970610	US 1995-389668	19950216 <--
WO 9531543	A1	19951123	WO 1995-US5768	19950509 <--
W: CA, JP, US, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: US 1994-242811 19940513
US 1995-389668 19950216
WO 1995-US5768 19950509
AB Complementary DNAs for the .beta.-subunit of bovine and human calcium- ***activated*** maxi-K ***potassium*** ***channel*** are cloned and expressed in animal cells. Cells presenting the protein, with or without the .alpha. subunit, may be used to screen for modulators of ***channel*** activity. Such modulators are useful in treating asthma, pregnant human myometrium, hypertension and angina, cerebral ischemia and in conditions where stimulation of neurotransmitter release is desired such as Alzheimer's disease and stimulation of damaged nerves. A cDNA was cloned by screening a bovine aortic smooth muscle cDNA library in .lambda.gt10 with amino acid sequence-derived oligonucleotide probes. The cRNA for the .alpha. and .beta. subunits of mammalian maxi-K channels injected into Xenopus oocytes led to the development of a ***potassium*** ***channel*** with the expected properties.

L7 ANSWER 52 OF 55 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:590774 HCAPLUS
DOCUMENT NUMBER: 127:289623
TITLE: ***Channel*** properties of carrier proteins. Functional studies of mitochondrial aspartate/glutamate carrier and phosphate carrier
AUTHOR(S): Herick, Klaus
CORPORATE SOURCE: Institut Biotechnologie, Forschungszentrum Julich G.m.b.H., Juelich, D-52425, Germany
SOURCE: Ber. Forschungszent. Juelich (***1997***), Juel-3384, 1-148 pp.
CODEN: FJBEE5; ISSN: 0366-0885
DOCUMENT TYPE: Report
LANGUAGE: German
AB ***Channel*** characteristics of an aspartate/glutamate carrier (AGC) from bovine heart mitochondria and a mitochondrial phosphate carrier (PIC) from Saccharomyces ***cerevisiae*** were investigated by biochem. and electrophys. methods. The strictly linked substrate-specific exchange function of the carrier was transformed reversibly in an unidirectional transport function by mercaptide formation of certain Cys residues. The uniport (efflux) is distinguished by a markedly decreased substrate specificity, which points to a ***channel*** -like mode of transport. The efflux was investigated in the reconstituted system with regard to the division of transport proteins into the extrema carrier and ***channel*** . The Hg-induced uniport of nonphysiol. substrates had the same ***activation*** energy (Ea) as the physiol. antiport function of the AGC. The value of Ea in the range of 63-89 kJ/mol indicated a carrier-mediated transport. The internal binding center of the AGC showed only a rest of specificity. The antiport rates were greater than the uniport rates, but the mol. activities of the efflux exceeded the antiport, which pointed to ***channel*** -mediated transport. The external binding center retained its specificity because only the efflux of anionic substrates could be ***inhibited*** selectively by external aspartate or glutamate. This trans- ***inhibition*** is assumed as a carrier characteristic. Beside the mercaptide formation of 2 Cys in the AGC, increase in the membrane voltage also caused an unidirectional transport function with the same kinetic characteristics. With electrophysiol. methods ***channel*** functions were measured in the unmodified reconstituted AGC/AAC (ADP/ATP carrier) protein fraction and the reconstituted AAC with conductivities of 20-200 pS. This measurements gave evidence for the existence of an intrinsic ***channel*** in the carrier protein. The PIC also showed a ***channel*** function without specific mercaptide formation. It was functionally expressed in the cytoplasm membrane of Xenopus laevis.

L7 ANSWER 53 OF 55 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:842668 HCAPLUS
DOCUMENT NUMBER: 123:220300
TITLE: Heterologous G protein coupled receptors expressed in yeast, their fusion with G proteins and use thereof in bioassay
INVENTOR(S): Pausch, Mark Henry; Ozenberger, Bradley Alton; Hadcock, John Richard; Price, Laura Alicia; Kajkowski, Eileen Marie; Kirsch, Donald Richard; Chaleff, Deborah Tardy
PATENT ASSIGNEE(S): American Cyanamid Co., USA
SOURCE: PCT Int. Appl., 121 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9521925	A1	19950817	WO 1995-US2075	19950214 <--
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5691188	A	19971125	US 1994-195729	19940214 <--
CA 2183166	AA	19950817	CA 1995-2183166	19950214 <--
AU 9518469	A1	19950829	AU 1995-18469	19950214 <--
EP 745130	A1	19961204	EP 1995-910301	19950214 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
JP 09510087	T2	19971014	JP 1995-521439	19950214 <--

PRIORITY APPLN. INFO.: US 1994-195729 19940214
WO 1995-US2075 19950214

AB The present invention is directed to expression vectors and yeast cells transformed therewith contg. a first heterologous nucleotide sequence which codes for a G protein-coupled receptor, for example, the somatostatin receptor, and a second nucleotide sequence which codes for all or a portion of a G protein .alpha..beta..gamma. complex. Said heterologous protein is phys. expressed in a host cell membrane in proper orientation for both stereoselective binding of ligands, as well as functional interaction with G proteins on the cytoplasmic side of the cell membrane. In some embodiments, a nucleotide sequence encoding a heterologous or chimeric G.alpha. protein is expressed in conjunction with nucleotide sequences from the yeast G protein .beta..gamma. subunits. A second aspect of the present invention provides expression vectors and yeast cells transformed therewith encoding chimeric yeast/heterologous G protein coupled receptors. A third aspect of the present invention is directed to methods of assaying compds. using such expression constructs and yeast cell expression systems to det. the effects of ligand binding to the heterologous receptors expressed in the systems.

L7 ANSWER 54 OF 55 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:591371 HCAPLUS
DOCUMENT NUMBER: 123:2770
TITLE: A strain of Saccharomyces ***cerevisiae*** expressing the gene encoding ***potassium*** transporter minK
INVENTOR(S): Kurtz, Stephen E.; Knickerbocker, Aron M.; Mccullogh, John R.
PATENT ASSIGNEE(S): Bristol-Myers Squibb Co., USA
SOURCE: Eur. Pat. Appl., 43 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 641860	A2	19950308	EP 1994-112995	19940819 <--
EP 641860	A3	19961227		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
US 5620892	A	19970415	US 1993-118101	19930907 <--
CA 2117616	AA	19950308	CA 1994-2117616	19940829 <--
JP 07163335	A2	19950627	JP 1994-213529	19940907 <--

PRIORITY APPLN. INFO.: US 1993-118101 19930907

AB A modified Saccharomyces ***cerevisiae*** cell is provided wherein the cell expresses minK but does not express TRK1 and TRK2. Also disclosed is a process for detecting modulators of minK, which comprises (a) treating such modified Saccharomyces ***cerevisiae*** cells with a test compd., (b) assessing growth in the presence of a test compd., and (c) detg. an increase or decrease in ***potassium*** uptake into the Saccharomyces ***cerevisiae*** cells. MinK ***inhibitors*** are useful anti-arrhythmic, antifibrillatory, or anti-ischemic agents. Thus, portions of genes TRK1 and TRK2 (for the yeast high and low affinity ***potassium*** transporters) were deleted and replaced with marker genes HIS3 or TRP1, resp. The double mutant (trk1-,trk2-) strain was obtained, exhibiting both high and low affinity ***potassium*** requirement phenotype, and transformed with plasmid pGal contg. either the wild type TRK1 gene or the heterologous human minK gene (which codes for a human ***potassium*** ***channel*** with very slow ***activation*** and inactivation kinetics). The resulting yeast strain carrying the plasmid pGal:HminK grew at 0.1 mM KCl, and ***potassium*** uptake was blocked by clofilium and dofetilide.

L7 ANSWER 55 OF 55 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:473196 HCAPLUS
DOCUMENT NUMBER: 111:73196
TITLE: Incorporation of ionic channels from yeast plasma membranes into black lipid membranes
AUTHOR(S): Gomez-Lagunas, F.; Pena, A.; Lievano, A.; Darszon, A.
CORPORATE SOURCE: Inst. Fisiol. Cel., Univ. Nac. Auton. Mexico, Mexico City, Mex.
SOURCE: Biophys. J. (***1989***), 56(1), 115-19
CODEN: BIOJAU; ISSN: 0006-3495
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The fusion of purified plasma membranes into planar bilayers allowed the study of yeast ion channels. The main cationic conductances detected were of 64 and 116 pS; however, larger and smaller conductances were also obsd. The 2 main conductances were sensitive to the K+ ***channel*** ***blockers*** , tetraethylammonium and Ba2+. Biionic expts. indicated that both conductances were K+-selective.

=> s potassium and tok1
L1 88 POTASSIUM AND TOK1

=> s trk1 and trk2 and tok1
L2 9 TRK1 AND TRK2 AND TOK1

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3 4 DUP REM L2 (5 DUPLICATES REMOVED)

=> d l3 ibib abs 1-3

L3 ANSWER 1 OF 4 WPIDS (C) 2002 THOMSON DERWENT
DUPLICATE 1
ACCESSION NUMBER: 2001-442137 [47] WPIDS
DOC. NO. CPI: C2001-133737
TITLE: Identifying inhibitors and activators of eukaryotic potassium channels, for use as pharmaceuticals, comprises using yeast cells that express heterologous, but no endogenous, potassium channels.
DERWENT CLASS: B04 D16
INVENTOR(S): LEBERER, E; LEEUW, T; RITSCHER, A
PATENT ASSIGNEE(S): (AVET) AVENTIS PHARMA DEUT GMBH
COUNTRY COUNT: 93
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001051519	A2	20010719	(200147)*	GE	78

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM
DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE
SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
DE 10000651 A1 20010726 (200150)
AU 2001057898 A 20010724 (200166)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001051519	A2	WO 2001-EP55	20010105
DE 10000651	A1	DE 2000-10000651	20000111
AU 2001057898	A	AU 2001-57898	20010105

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001057898	A Based on	WO 200151519

PRIORITY APPLN. INFO: DE 2000-10000651 20000111
AN 2001-442137 [47] WPIDS
AB WO 200151519 A UPAB: 20010822
NOVELTY - Identifying inhibitors or activators (A) of a eukaryotic potassium channel (KC) by applying a test compound to a mutant Saccharomyces cerevisiae cell in which:
(i) the three endogenous KC (***TRK1*** , ***TRK2*** and ***TOK1***) are not expressed; but
(ii) a eukaryotic KC is expressed heterologously, where the effect of the compound on the eukaryotic KC is then determined.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) mutant S. cerevisiae cells in which the three endogenous KC are not expressed;
(2) preparing cells of (1) by knockout destruction of the relevant genes;
(3) similar method for identifying activators of eukaryotic KC in which the cells are treated with both a test compound and a known inhibitor of KC;
(4) test kits containing the cells of (1); and
(5) preparation of pharmaceuticals using (A) identified by the new methods.
ACTIVITY - None given.
MECHANISM OF ACTION - Potassium channel activator or inhibitor..
USE - The method is used to identify inhibitors or activators (A) of a eukaryotic potassium channel (claimed). (A) are potentially useful as pharmaceuticals (no more details).
ADVANTAGE - The method is easily automated for parallel processing of many samples, using either different concentrations of test compounds and/or different levels of heterologous gene expression. It allows identification of compounds that inhibit human KC selectively.
Dwg.0/14

L3 ANSWER 2 OF 4 WPIDS (C) 2002 THOMSON DERWENT
DUPLICATE 2
ACCESSION NUMBER: 2001-603577 [69] WPIDS
DOC. NO. CPI: C2001-179031
TITLE: Genetically modified yeast lacking endogenous potassium transport activity, useful for identifying e.g. antiarrhythmic agents, includes a functional human potassium channel.
DERWENT CLASS: B04 D16
INVENTOR(S): LICHTENBERG-FRATE, H; LUDWIG, J
PATENT ASSIGNEE(S): (LICH-I) LICHTENBERG-FRATE H; (LUDW-I) LUDWIG J
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
DE 19953478	A1	20011011	(200169)*		40

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
DE 19953478	A1	DE 1999-19953478	19991106

PRIORITY APPLN. INFO: DE 1999-19953478 19991106
AN 2001-603577 [69] WPIDS
AB DE 19953478 A UPAB: 20011126
NOVELTY - Genetically modified Saccharomyces cerevisiae (A) in which (i) the endogenous potassium-translocation systems (***TRK1*** , ***TRK2*** and ***TOK1***) are specifically deleted and (ii) the human erg potassium ion channel (HERG) is stably integrated and expressed, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
S. cerevisiae mutants with defects in potassium uptake as a result of mutation of the ***TRK1*** /2 and ***TOK1*** genes, by introducing one or more selection markers (for auxotrophy or resistance);
(1) stably integrating human potassium ion channel genes for expression in the genome of a S. cerevisiae host that lacks functional ***TRK1*** /2 and ***TOK1*** ; and
(2) identifying specific modulators of HERG.
ACTIVITY - Antiarrhythmic; antiinflammatory.
MECHANISM OF ACTION - Blocking or activating potassium channels.
USE - (A) are used for identifying specific modulators of HERG, potentially useful as antiarrhythmic, antifibrillatory and antiinflammatory agents.
ADVANTAGE - (A) are easier to culture than animals cells normally used; are genetically well characterized and are suitable for screening substance libraries in microtiter plates.
Dwg.0/11

L3 ANSWER 3 OF 4 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-442135 [47] WPIDS
DOC. NO. CPI: C2001-133735
TITLE: Identifying inhibitors and activators of eukaryotic potassium channels, for use as therapeutic agents,

comprises using a transformed yeast cell that does not express endogenous channels.
DERWENT CLASS: B04 D16
INVENTOR(S): GISSMANN, L; MICHEL, N; MUELLER, M; OSEN, W; ZENTGRAF, H
PATENT ASSIGNEE(S): (DEKR-N) DEUT KREBSFORSCHUNGSZENTRUM
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001051516	A2	20010719	(200147)*	GE	78

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DK DM DZ
EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK
LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG
SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
DE 10001230 A1 20010802 (200151)
AU 2001040424 A 20010724 (200166)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001051516	A2	WO 2001-DE134	20010115
DE 10001230	A1	DE 2000-10001230	20000113
AU 2001040424	A	AU 2001-40424	20010115

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001040424	A Based on	WO 200151516

PRIORITY APPLN. INFO: DE 2000-10001230 20000113
AN 2001-442135 [47] WPIDS
AB WO 200151516 A UPAB: 20010822
NOVELTY - Identifying inhibitors (A) or activators (B) of a eukaryotic potassium channel by measuring the effect of a test compound on a mutant Saccharomyces cerevisiae that:
(i) does not express the endogenous potassium channels ***TRK1***, ***TRK2*** and ***TOK1*** ; but
(ii) does express a heterologous eukaryotic potassium channel (I).
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(a) mutant S. cerevisiae cell that does not express the specified endogenous channels;
(b) producing cells of (a) by knockout of the RTK1, ***TRK2*** and ***TOK1*** genes;
(c) identifying (B) by measuring the effect of test compounds on the mutant S. cerevisiae in presence of a known inhibitor;
(d) a test kit containing the cells of (a); and
(e) preparation of a pharmaceutical containing (A) or (B).
ACTIVITY - None given.
MECHANISM OF ACTION - Potassium channel inhibitor or activator.
USE - The method is used to identify substances that inhibit/activate human potassium channels selectively, potentially useful as therapeutic agents, and also to detect toxic compounds.
ADVANTAGE - The method is well suited for automation and for performing many analyzes in parallel.
Dwg.0/14

=> d l3 ibib abs 4

L3 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
ACCESSION NUMBER: 1999:212609 BIOSIS
DOCUMENT NUMBER: PREV199900212609
TITLE: Potassium uptake through the ***TOK1*** K+ channel in the budding yeast.
AUTHOR(S): Fairman, C.; Zhou, X.-L.; Kung, C. (1)
CORPORATE SOURCE: (1) Laboratory of Molecular Biology, University of Wisconsin, 1525 Linden Dr., Madison, WI, 53706 USA
SOURCE: Journal of Membrane Biology, (March 15, 1999) Vol. 168, No. 2, pp. 149-157.
ISSN: 0022-2631.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The current through ***TOK1*** (YKC1), the outward-rectifying K+ channel in Saccharomyces cerevisiae, was amplified by expressing ***TOK1*** from a plasmid driven by a strong constitutive promoter. ***TOK1*** so hyper-expressed could overcome the K+ auxotrophy of a mutant missing the two K+ transporters, ***TRK1*** and ***TRK2*** .
This trk1DELTA.trk2DELTA double mutant hyperexpressing the ***TOK1*** transgene had a higher internal K+ content than one expressing the empty plasmid. We examined protoplasts of these ***TOK1*** -hyperexpressing cells under a patch clamp. Besides the expected K+ outward current activating at membrane potential (Vm) above the K+ equilibrium potential (EK+), a small inward current was consistently observed when the Vm was slightly below EK+. The inward and the outward currents are similar in their activation rates, deactivation rates, ion specificities and Ba2+ inhibition, indicating that they flow through the same channel. Thus, the yeast outwardly rectifying K+ channel can take up K+ into yeast cells, at least under certain conditions.

=> s ykc1 and trk1 and trk2
L4 4 YKC1 AND TRK1 AND TRK2

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 1 DUP REM L4 (3 DUPLICATES REMOVED)

=> s l5 not l3
L6 0 L5 NOT L3

=> s ykc1 and potassium
L7 21 YKC1 AND POTASSIUM

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 6 DUP REM L7 (15 DUPLICATES REMOVED)

=> s l8 not l3
L9 5 L8 NOT L3

=> d l9 ibib abs 1-5

L9 ANSWER 1 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:484291 BIOSIS
DOCUMENT NUMBER: PREV199900484291
TITLE: Mutations in the yeast two pore K+ channel ***YKC1*** identify functional differences between the pore domains.
AUTHOR(S): Vergani, Paola; Blatt, Michael R. (1)
CORPORATE SOURCE: (1) Laboratory of Plant Physiology and Biophysics, University of London, London UK
SOURCE: FEBS Letters, (Sept. 24, 1999) Vol. 458, No. 3, pp. 285-291.
ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The K+ channel of Saccharomyces cerevisiae encoded by the ***YKC1*** gene includes two pore-loop sequences that are thought to form the hydrophilic lining of the pore. Gating of the channel is promoted by

membrane depolarisation and is regulated by the extracellular K+ concentration ((K+)o) both in the yeast and when expressed in Xenopus oocytes. Our previous work showed that substitutions of equivalent residues L293 and A428 within the pore-loops had qualitatively similar effects on both the (K+)o-sensitivity of channel gating and its voltage-dependence. Here, we report that mutations of equivalent residues N275 and N410, N-terminal from the K+ channel signature sequences of the two pores, have very different actions on channel gating and, in this case, are without effect on its voltage-sensitivity. The mutation N410D slowed current activation in a (K+)o-dependent manner and it accelerated deactivation, but without significant effect on the apparent affinity for K+. The N275D mutant, by contrast, had little effect on the (K+)o-sensitivity for activation and it greatly altered the (K+)o-dependence of current deactivation. Neither mutant affected the voltage-dependence of the steady-state current nor the ability for other alkali cations to substitute for K+ in regulating gating. The double mutant N410D-N275D showed characteristics of N410D in the (K+)o-sensitivity of current activation and of N275D in the (K+)o-sensitivity of deactivation, suggesting that little interaction occurs between pore domains with mutations at these sites. The results indicate that the two pore domains are not functionally equivalent and they suggest that the regulation of gating by external K+ is mediated by K+ binding at two physically distinct sites with different actions.

L9 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:186431 BIOSIS
DOCUMENT NUMBER: PREV199900186431
TITLE: Rectification of the yeast K+ channel, ***Ykc1*** (Tok1.
AUTHOR(S): Loukin, S. H. (1); Kung, C. (1); Saimi, Y. (1)
CORPORATE SOURCE: (1) Laboratory of Molecular Biology, Univ. of Wisconsin, Madison, WI, 53706 USA
SOURCE: Biophysical Journal, (Jan., 1999) Vol. 76, No. 1 PART 2, pp. A415.
Meeting Info.: Forty-third Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 13-17, 1999
ISSN: 0006-3495.
DOCUMENT TYPE: Conference
LANGUAGE: English

L9 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:57903 BIOSIS
DOCUMENT NUMBER: PREV199900057903
TITLE: Mutations in the pore regions of the yeast K+ channel ***YKC1*** affect gating by extracellular K.
AUTHOR(S): Vergani, Paola (1); Hamilton, David; Jarvis, Simon; Blatt, Michael R.
CORPORATE SOURCE: (1) Lab. Plant Physiol. Biophysics, Univ. London, Wye Coll., Wye, Kent TN25 5AH UK
SOURCE: EMBO (European Molecular Biology Organization) Journal, (Dec. 15, 1998) Vol. 17, No. 24, pp. 7190-7198.
ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The product of the Saccharomyces cerevisiae K+-channel gene ***YKC1*** includes two pore-loop sequences that are thought to form the hydrophilic lining of the pore. Gating of the channel is promoted by membrane depolarization and is regulated by extracellular K+ concentration ((K+)o) both in the yeast and when expressed in Xenopus oocytes. Analysis of the wild-type current now shows that: (i) (K+)o suppresses a very slowly relaxing component, accelerating activation; (ii) (K+)o slows deactivation in a dose-dependent fashion; and (iii) Rb+, Cs+ and, to a lesser extent, Na+ substitute for K+ in its action on gating. We have identified single residues, L293 and A428, at equivalent positions within the two pore loops that affect the (K+)o sensitivity. Substitution of these residues gave channels with reduced sensitivity to (K+)o in macroscopic current kinetics and voltage dependence, but had only minor effects on selectivity among alkali cations in gating and on single-channel conductance. In some mutants, activation was slowed sufficiently to confer a sigmoidicity to current rise at low (K+)o. The results indicate that these residues are involved in (K+)o sensing. Their situation close to the permeation pathway points to an interaction between gating and permeation.

L9 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:435978 BIOSIS
DOCUMENT NUMBER: PREV199799735181
TITLE: Random mutagenesis reveals a region important for gating of the yeast K+ channel ***Ykc1*** .
AUTHOR(S): Loukin, Stephen H.; Vaillant, Brian; Zhou, Xin-Liang; Spalding, Edgar P.; Kung, Ching; Saimi, Yoshiro (1)
CORPORATE SOURCE: (1) Lab. Mol. Biol., Univ. Wisconsin, Madison, WI 53706 USA
SOURCE: EMBO (European Molecular Biology Organization) Journal, (1997) Vol. 16, No. 16, pp. 4817-4825.
ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
AB ***YKC1*** (TOK1, DUK1, YORK) encodes the outwardly rectifying K+ channel of the yeast plasma membrane. Non-targeted mutations of ***YKC1*** were isolated by their ability to completely block proliferation when expressed in yeast. All such mutations examined occurred near the cytoplasmic ends of the transmembrane segments following either of the duplicated P loops, which we termed the 'post-P loop' (PP) regions. These PP mutations specifically caused marked defects in the 'C-1' states, a set of interrelated closed states that ***Ykc1*** enters and exits at rates of tens to hundreds of milliseconds. These results indicate that the ***Ykc1*** PP region plays a role in determining closed state conformations and that non-targeted mutagenesis and microbial selection can be a valuable tool for probing structure-function relationships of ion channels.

L9 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:539759 BIOSIS
DOCUMENT NUMBER: PREV199598554059
TITLE: YKJC1 encodes the depolarization-activated K+ channel in the plasma membrane of yeast.
AUTHOR(S): Zhou, Xin-Liang; Vaillant, Brian; Loukin, Stephen H.; Kung, Ching (1); Saimi, Yoshiro
CORPORATE SOURCE: (1) Lab. Molecular Biol., Univ. Wisconsin, Madison, WI 53706 USA
SOURCE: FEBS Letters, (1995) Vol. 373, No. 2, pp. 170-176.
ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Our previous patch-clamp studies showed that depolarization activates a K+-specific current in the plasma membrane of the budding yeast, Saccharomyces cerevisiae (Gustin et a). (1986) Science 233, 1195-1197). The Yeast Genome Sequencing Project has now uncovered on the left arm of chromosome X an open reading frame (ORF) that predicts a 77-kDa protein reminiscent of a shaker-like alpha subunit with 6 membrane spans followed by a subunit with 2 spans. We found that deleting this ORF removes the yeast K+ current. Furnishing the ORF from plasmids restores or even greatly amplifies this current. These manipulations have no effects on the 40-pS mechanosensitive conductance also native to this membrane. Thus, this ORF, named ***YKC1*** here, likely encodes a structure for the K+-specific channel of the yeast plasma membrane. This and other K+ channel subunits are compared and the possible uses of this gene in research are discussed. ***YKC1*** has recently been shown by others to induce in frog oocytes a K+ current. Its activation is coupled to E-K+ and its outward rectification depends on external divalent cations. We found the ***YKC1*** channel in its native membrane activates at low voltages largely independent of E-K+ and it remains so despite removal of divalents by chelation.

=> s ykc1 or tok1 or duk1 or york
L10 82939 YKC1 OR TOK1 OR DUK1 OR YORK

=> s l10 and potassium
L11 726 L10 AND POTASSIUM

=> s trk1 and trk2
L12 149 TRK1 AND TRK2

=> s l11 and l12
L13 9 L11 AND L12

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14 4 DUP REM L13 (5 DUPLICATES REMOVED)

=> s l14 not l3
L15 0 L14 NOT L3

=> s inhibit? or activat? or agonist or antagonist
2 FILES SEARCHED...
L16 6823088 INHIBIT? OR ACTIVAT? OR AGONIST OR ANTAGONIST

=> s l11 and l16

L17 211 L11 AND L16

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18 108 DUP REM L17 (103 DUPLICATES REMOVED)

=> s l18 and py<2000
1 FILES SEARCHED...
3 FILES SEARCHED...
4 FILES SEARCHED...
L19 86 L18 AND PY<2000

=> d l19 ibib abs 1-86

L19 ANSWER 1 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:91089 BIOSIS
DOCUMENT NUMBER: PREV200000091089
TITLE: Correlations between skeletal muscle mass and bone mass in children 6-18 years: Influences of sex, ethnicity, and pubertal status.
AUTHOR(S): Wang, Jack (1); Horlick, Mary; Thornton, John C.; Levine, Lenore S.; Heymsfield, Steven B.; Pierson, Richard N., Jr.
CORPORATE SOURCE: (1) Body Composition Unit, St. Lukes-Roosevelt Hospital Center, 1111 Amsterdam Avenue, New York, NY, 10025 USA
SOURCE: Growth Development and Aging, (***Autumn, 1999***) Vol. 63, No. 3, pp. 99-109.
ISSN: 1041-1232.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A constant sex-specific relationship between skeletal muscle mass and bone mass was observed in healthy adults based on TBK/TBCa, using TBK (total body ***potassium***) by 40K counting and TBCa (total body calcium) by in-vivo neutron ***activation*** analysis (Ellis and Cohn, 1975). We revisited this topic in children by studying correlations between TBK and TBCa, and by comparing TBK/TBCa between sexes, pubertal groups (prepubertal and pubertal) and ethnic groups in 141 white, 101 black, and 62 Asian healthy children, aged 6 - 18 years, living in New ***York*** City. TBK was measured by 40K counting, and TBCa by dual energy x-ray absorptiometry. TBK and TBCa were significantly correlated from 6 to 18 years ($r > 0.93$), but the correlation equations varied by gender and ethnicity. Boys had significantly more TBK and greater TBK/TBCa than girls at a given age and weight, reflecting greater skeletal muscle mass in boys from 6 years, the age at which the study started. TBK/TBCa in blacks was significantly smaller than whites and Asians in both sexes in prepuberty and puberty, and pubertal black girls had the smallest mean TBK/TBCa. No significant differences were found between whites and Asians. TBK/TBCa decreased as body weight increased in prepubertal girls, and decreased as body weight and age increased in pubertal girls, but did not change with body weight or age in boys of any subgroup. The inverse relationship between TBK/TBCa and age in pubertal girls suggests greater increase in TBCa compared to TBK than in other groups, while the constant TBK/TBCa in boys reflects proportional increases in TBK and TBCa. Thus TBK/TBCa can be used as an index of relative growth in skeletal muscle mass and bone mass in white, black, and Asian children according to sex, age and pubertal status.

L19 ANSWER 2 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:61230 BIOSIS
DOCUMENT NUMBER: PREV200000061230
TITLE: K+-dependent composite gating of the yeast K+ channel, ***TOK1*** .
AUTHOR(S): Loukin, Stephen H. (1); Saimi, Yoshiro
CORPORATE SOURCE: (1) Laboratory of Molecular Biology, University of Wisconsin, 1525 Linden Dr., Madison, WI USA
SOURCE: Biophysical Journal, (***Dec., 1999***) Vol. 77, No. 6, pp. 3060-3070.
ISSN: 0006-3495.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB ***TOK1*** encodes an outwardly rectifying K+ channel in the plasma membrane of the budding yeast Saccharomyces cerevisiae. It is capable of dwelling in two kinetically distinct impermeable states, a near-instantaneously ***activating*** R state and a set of related delayed ***activating*** C states (formerly called C2 and C1, respectively). Dwell in the R state is dependent on membrane potential and both internal and external K+ in a manner consistent with the K+ electrochemical potential being its determinant, where dwell in the C states is dependent on voltage and only external K+. Whereas ***activation*** from the C states showed high temperature dependencies, typical of gating transitions in other Shaker-like channels, ***activation*** from the R state had a temperature dependence nearly as low as that of simple ionic diffusion. These findings lead us to conclude that although the C states reflect the activity of an internally oriented channel gate, the R state results from an intrinsic gating property of the channel filter region.

L19 ANSWER 3 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:2530 BIOSIS
DOCUMENT NUMBER: PREV200000002530
TITLE: A molecular target for viral killer toxin: ***TOK1*** ***potassium*** channels.
AUTHOR(S): Ahmed, Aamir; Sesti, Federico; Ilan, Nitza; Shih, Theodore M.; Sturley, Stephen L.; Goldstein, Steve A. N. (1)
CORPORATE SOURCE: (1) Departments of Pediatrics and Cellular and Molecular Physiology, Boyer Center for Molecular Medicine Yale University School of Medicine, New Haven, CT, 06536 USA
SOURCE: Cell, (***Oct. 29, 1999***) Vol. 99, No. 3, pp. 283-291.
ISSN: 0092-8674.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Killer strains of S. cerevisiae harbor double-stranded RNA viruses and secrete protein toxins that kill virus-free cells. The K1 killer toxin acts on sensitive yeast cells to perturb ***potassium*** homeostasis and cause cell death. Here, the toxin is shown to ***activate*** the

plasma membrane ***potassium*** channel of S. cerevisiae, ***TOK1***
. Genetic deletion of ***TOK1*** confers toxin resistance; overexpression increases susceptibility. Cells expressing ***TOK1*** exhibit toxin-induced ***potassium*** flux; those without the gene do not. K1 toxin acts in the absence of other viral or yeast products: toxin synthesized from a cDNA increases open probability of single ***TOK1***
channels (via reversible destabilization of closed states) whether channels are studied in yeast cells or X. laevis oocytes.

L19 ANSWER 4 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:484291 BIOSIS
DOCUMENT NUMBER: PREV199900484291
TITLE: Mutations in the yeast two pore K+ channel ***YKC1*** identify functional differences between the pore domains.
AUTHOR(S): Vergani, Paola; Blatt, Michael R. (1)
CORPORATE SOURCE: (1) Laboratory of Plant Physiology and Biophysics,
University of London, London UK
SOURCE: FEBS Letters, (***Sept: 24, 1999***) Vol. 458, No. 3, pp. 285-291.
ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The K+ channel of Saccharomyces cerevisiae encoded by the ***YKC1***
gene includes two pore-loop sequences that are thought to form the hydrophilic lining of the pore. Gating of the channel is promoted by membrane depolarisation and is regulated by the extracellular K+ concentration ((K+)o) both in the yeast and when expressed in Xenopus oocytes. Our previous work showed that substitutions of equivalent residues L293 and A428 within the pore-loops had qualitatively similar effects on both the (K+)o-sensitivity of channel gating and its voltage-dependence. Here, we report that mutations of equivalent residues N275 and N410, N-terminal from the K+ channel signature sequences of the
two pores, have very different actions on channel gating and, in this case, are without effect on its voltage-sensitivity. The mutation N410D slowed current ***activation*** in a (K+)o-dependent manner and it accelerated deactivation, but without significant effect on the apparent affinity for K+. The N275D mutant, by contrast, had little effect on the (K+)o-sensitivity for ***activation*** and it greatly altered the (K+)o-dependence of current deactivation. Neither mutant affected the voltage-dependence of the steady-state current nor the ability for other alkali cations to substitute for K+ in regulating gating. The double mutant N410D-N275D showed characteristics of N410D in the (K+)o-sensitivity of current ***activation*** and of N275D in the (K+)o-sensitivity of deactivation, suggesting that little interaction occurs between pore domains with mutations at these sites. The results indicate that the two pore domains are not functionally equivalent and they suggest that the regulation of gating by external K+ is mediated by K+ binding at two physically distinct sites with different actions.

L19 ANSWER 5 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:452207 BIOSIS
DOCUMENT NUMBER: PREV199900452207
TITLE: Localized amyloidosis of the seminal vesicle: Possible association with hormonally treated prostatic adenocarcinoma.
AUTHOR(S): Unger, Pamela D. (1); Wang, Qi; Gordon, Ronald E.; Stock,
Richard; Stone, Nelson
CORPORATE SOURCE: (1) Department of Pathology, Mount Sinai Hospital, One
Gustave L. Levy Pl, New York, NY, 10029 USA
SOURCE: Archives of Pathology & Laboratory Medicine, (***Dec.,***
*** 1997***) Vol. 121, No. 12, pp. 1265-1268.
ISSN: 0363-0153.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Objective.-Localized seminal vesicle amyloidosis is an unusual finding in
surgical pathology material. Previous studies have demonstrated that the amyloid is directly produced by the seminal vesicle epithelial cells. We investigated the possible association of seminal vesicle amyloid in patients hormonally treated for prostate carcinoma. Methods.-Cases were collected from over 200 prostate needle biopsies, seminal vesicle biopsies, and prostatectomy specimens from the surgical pathology files at The Mount Sinai Hospital, New ***York*** , NY. None of the patients with seminal vesicle amyloidosis had a chronic inflammatory disorder, serum or urine protein abnormalities, or other identifiable masses. Results.-Six cases of localized seminal vesicle amyloidosis were found in the surgical pathology material examined. Five of the six cases had prostatic carcinoma, and one case was seen in a biopsy for benign prostatic hyperplasia. Four of the five carcinoma cases had prior hormonal treatment (luteinizing hormone-releasing hormone ***agonist*** with an
antiandrogen agent, and one patient, in addition, had received radiotherapy). The amyloid deposits were limited to the seminal vesicle lamina propria without involvement of vascular walls. The amyloid reacted
with Congo red staining that was sensitive to ***potassium*** permanganate. Immunohistochemically, all cases were negative for AA amyloid, beta2-microglobulin, and kappa and lambda light chains. Conclusion.-We raise the possibility that in some instances, prior hormonal therapy may act as a seminal vesicle epithelial stimulant for the elaboration of this protein.

L19 ANSWER 6 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:443095 BIOSIS
DOCUMENT NUMBER: PREV199900443095
TITLE: Overexpression of the volatile anesthetic- ***activated*** baseline K+ channel ***TOK1*** ***inhibits*** yeast growth.
AUTHOR(S): Yost, Charles S. (1); O'Rourke, Sean M. (1); Sampson, Elizabeth R. (1); Herskowitz, Ira (1)
CORPORATE SOURCE: (1) University of California San Francisco, San Francisco,
CA USA
SOURCE: Anesthesiology (Hagerstown), (***Sept., 1999***) Vol. 91, No. 3A, pp. A355.
Meeting Info.: Annual Meeting of the American Society of Anesthesiologists Dallas, Texas, USA October 9-13, 1999 American Society of Anesthesiologists
. ISSN: 0003-3022.
DOCUMENT TYPE: Conference
LANGUAGE: English

L19 ANSWER 7 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:425729 BIOSIS
DOCUMENT NUMBER: PREV199900425729
TITLE: Local anesthetic ***inhibition*** of baseline ***potassium*** channels with two pore domains in tandem.
AUTHOR(S): Kindler, Christoph H.; Yost, C. Spencer; Gray, Andrew T.
(1)
CORPORATE SOURCE: (1) Department of Anesthesia, University of California, San
Francisco, 513 Parnassus Avenue, San Francisco, CA, 94143-0542 USA
SOURCE: Anesthesiology (Hagerstown), (***April, 1999***) Vol. 90, No. 4, pp. 1092-1102.
ISSN: 0003-3022.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Background: Recently, a new structural family of ***potassium*** channels characterized by two pore domains in tandem within their primary
amino acid sequence was identified. These tandem pore domain ***potassium*** channels are not gated by voltage and appear to be involved in the control of baseline membrane conductances. The goal of this study was to identify mechanisms of local anesthetic action on these channels. Methods: Oocytes of Xenopus laevis were injected with cRNA from
five cloned tandem pore domain baseline ***potassium*** channels (TASK, TREK-1, ***TOK1*** , ORK1, and TWIK-1), and the effects of

several local anesthetics on the heterologously expressed channels were assayed using two-electrode voltage-clamp and current-clamp techniques. Results: Bupivacaine (1 mM) ***inhibited*** all studied tandem pore ***potassium*** channels, with TASK ***inhibited*** most potently.

The potency of ***inhibition*** was directly correlated with the octanol: buffer distribution coefficient of the local anesthetic, with the exception of tetracaine, to which TASK is relatively insensitive. The approximate 50% ***inhibitory*** concentrations of TASK were 709 muM

mepivacaine, 222 muM lidocaine, 51 muM R(+)-ropivacaine, 53 muM S(-)-ropivacaine, 668 muM tetracaine, 41 muM bupivacaine, and 39 muM etidocaine. Local anesthetics (1 mM) significantly depolarized the resting membrane potential of TASK cRNA-injected oocytes compared with saline-injected control oocytes (tetracaine 22 +- 6 mV vs. 7 +- 1 mV, respectively, and bupivacaine 31 +- 7 mV vs. 6 +- 4 mV). Conclusions: Local anesthetics ***inhibit*** tandem pore domain baseline ***potassium*** channels, and they could depolarize the resting membrane

potential of cells expressing these channels. Whether ***inhibition*** of these channels contributes to conduction blockade or to the adverse effects of local anesthetics remains to be determined.

L19 ANSWER 8 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:212609 BIOSIS
DOCUMENT NUMBER: PREV199900212609
TITLE: ***Potassium*** uptake through the ***TOK1*** K+ channel in the budding yeast.
AUTHOR(S): Fairman, C.; Zhou, X.-L.; Kung, C. (1)
CORPORATE SOURCE: (1) Laboratory of Molecular Biology, University of
Wisconsin, 1525 Linden Dr., Madison, WI, 53706 USA
SOURCE: Journal of Membrane Biology, (***March 15, 1999***) Vol. 168, No. 2, pp. 149-157.
ISSN: 0022-2631.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The current through ***TOK1*** (***YKC1***), the outward-rectifying K+ channel in Saccharomyces cerevisiae, was amplified

by expressing ***TOK1*** from a plasmid driven by a strong constitutive promoter. ***TOK1*** so hyper-expressed could overcome

the K+ auxotrophy of a mutant missing the two K+ transporters, TRK1 and

TRK2. This trk1DELTA trk2DELTA double mutant hyperexpressing the ***TOK1*** transgene had a higher internal K+ content than one expressing the empty plasmid. We examined protoplasts of these ***TOK1*** -hyperexpressing cells under a patch clamp. Besides the expected K+ outward current ***activating*** at membrane potential (Vm) above the K+ equilibrium potential (EK+), a small inward current was

consistently observed when the Vm was slightly below EK+. The inward and

the outward currents are similar in their ***activation*** rates, deactivation rates, ion specificities and Ba2+ ***inhibition*** , indicating that they flow through the same channel. Thus, the yeast outwardly rectifying K+ channel can take up K+ into yeast cells, at least under certain conditions.

L19 ANSWER 9 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:57903 BIOSIS
DOCUMENT NUMBER: PREV199900057903
TITLE: Mutations in the pore regions of the yeast K+ channel ***YKC1*** affect gating by extracellular K.
AUTHOR(S): Vergani, Paola (1); Hamilton, David; Jarvis, Simon; Blatt,
Michael R.
CORPORATE SOURCE: (1) Lab. Plant Physiol. Biophysics, Univ. London, Wye
Coll., Wye, Kent TN25 5AH UK
SOURCE: EMBO (European Molecular Biology Organization) Journal, (
Dec. 15, 1998) Vol. 17, No. 24, pp. 7190-7198.
ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The product of the Saccharomyces cerevisiae K+-channel gene ***YKC1***

includes two pore-loop sequences that are thought to form the hydrophilic lining of the pore. Gating of the channel is promoted by membrane depolarization and is regulated by extracellular K+ concentration ((K+)o) both in the yeast and when expressed in Xenopus oocytes. Analysis of the wild-type current now shows that: (i) (K+)o suppresses a very slowly relaxing component, accelerating ***activation*** ; (ii) (K+)o slows deactivation in a dose-dependent fashion; and (iii) Rb+, Cs+ and, to a lesser extent, Na+ substitute for K+ in its action on gating. We have identified single residues, L293 and A428, at equivalent positions within the two pore loops that affect the (K+)o sensitivity. Substitution of these residues gave channels with reduced sensitivity to (K+)o in macroscopic current kinetics and voltage dependence, but had only minor effects on selectivity among alkali cations in gating and on single-channel conductance. In some mutants, ***activation*** was slowed sufficiently to confer a sigmoidicity to current rise at low (K+)o. The results indicate that these residues are involved in (K+)o sensing. Their situation close to the permeation pathway points to an interaction between gating and permeation.

L19 ANSWER 10 OF 86 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:262167 BIOSIS
DOCUMENT NUMBER: PREV199800262167
TITLE: ***TOK1*** is a volatile anesthetic stimulated K channel.
AUTHOR(S): Gray, Andrew T. (1); Winegar, Bruce D.; Leonoudakis, Dmitri
J.; Forsayeth, John R.; Yost, Spencer
CORPORATE SOURCE: (1) Anesth. Res. Laboratory, Room S261, Univ. California
Med. Cent., 513 Parnassus Ave., San Francisco, CA 94143-0542 USA
SOURCE: Anesthesiology (Hagerstown), (***April, 1998***) Vol. 88, No. 4, pp. 1076-1084.
ISSN: 0003-3022.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Background: Volatile anesthetic agents can ***activate*** the S channel, a baseline ***potassium*** (K+) channel, of the marine mollusk Aplysia. To investigate whether cloned ion channels with electrophysiologic properties similar to the S channel (***potassium*** selectivity, outward rectification, and ***activation*** independent of voltage) also are modulated by volatile anesthetic agents, the authors expressed the cloned yeast ion channel ***TOK1*** (tandem pore domain,

outwardly rectifying K+ channel) in Xenopus oocytes and studied its sensitivity to volatile agents. Methods: Standard two-electrode voltage and patch clamp recording methods were used to study ***TOK1*** channels expressed in Xenopus oocytes. Results: Studies with two-electrode

voltage clamp at room temperature showed that halothane, isoflurane, and desflurane increased ***TOK1*** outward currents by 48-65% in barium

Frog Ringer's perfusate. The concentrations at which 50% potentiation occurred (EC50. values) were in the range of 768-814 muM (0.016-0.044 atm)

and had a rank order of potency in atm in which halothane > isoflurane > desflurane. The potentiation of ***TOK1*** by volatile anesthetic agents was rapid and reversible (onset and offset, 1-20 s). In contrast, the nonanesthetic 1,2-dichlorohexafluorocyclobutane did not potentiate ***TOK1*** currents in concentrations up to five times the MAC value predicted by the Meyer-Overton hypothesis based on oil/gas partition coefficients. Single ***TOK1*** channel currents were recorded from excised outside-out patches. The single channel open probability increased as much as twofold in the presence of isoflurane and rapidly returned to the baseline values on washout. Volatile anesthetic agents did not alter the ***TOK1*** single channel current-voltage (I-V) relationship, however, suggesting that the site of action does not affect the permeation pathway of the channel. Conclusion: ***TOK1*** is a ***potassium***

channel that is stimulated by volatile anesthetic agents. The concentrations over which potentiation occurred (EC50 values) were higher

than those commonly used in clinical practice (approximately twice

09/758036
AH#13

562 0892
555 9026

1. Document ID: US 5795770 A

L5: Entry 1 of 4

File: USPT

Aug 18, 1998

US-PAT-NO: 5795770
DOCUMENT-IDENTIFIER: US 5795770 A

TITLE: Genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels and method to use the same

DATE-ISSUED: August 18, 1998

US-CL-CURRENT: 435/254.2; 435/254.4, 435/29, 435/320.1, 536/23.6

APPL-NO: 8/ 795788
DATE FILED: February 5, 1997

PARENT-CASE:

This is a continuation of application Ser. No. 07/923,094, filed Jul. 31, 1992, now abandoned which is a continuation-in-part of U.S. Ser. No. 874,846 filed April 27, 1992.

IN: Gaber; Richard F.

AB: This invention relates to genetically engineered eukaryotic organisms, such as yeast, that are made capable of detecting the expression of heterologous ion channels. These organisms include a potassium transport defective phenotype eukaryotic organism transformed with DNA that suppresses the potassium transport defective phenotype in the organism. A potassium transport gene is set out in Sequence Id. No. 1. This genetically engineered organism can be used to screen for new herbicides or drugs.

L5: Entry 1 of 4

File: USPT

Aug 18, 1998

DOCUMENT-IDENTIFIER: US 5795770 A
TITLE: Genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels and method to use the same

Detailed Description Paragraph Right (3):
It should be noted, however, that other potassium-transporting proteins, (not just those known to function as channels) could also suppress the potassium transport defect in trk1.DELTA. trk2.DELTA. cells since these proteins could also represent essential plant proteins and thus, be useful in the screening process for new herbicides or drugs. The KAT1 gene has the following characteristics: 1) KAT1 suppresses the Trk- phenotype of S. cerevisiae cells deleted for their endogenous potassium transporters; 2) the inferred protein sequence includes a cluster of six putative membrane-spanning domains and conserved amino acids sequences corresponding to the presumptive voltage-sensing (S4) and pore-forming (SS1-SS2 or H5) regions; and 3) potassium channel-specific blockers (TEA and Ba.sup.2+) inhibit of KAT1 in vivo. Alternatively, Schizosaccharomyces pombe, could be transformed with pKAT1 due to the presence of the Saccharomyces cerevisiae selectable marker URA3.

2. Document ID: JP 07163335 A

5620892

L5: Entry 2 of 4

File: JPAB

Jun 27, 1995

PUB-NO: JP407163335A
DOCUMENT-IDENTIFIER: JP 07163335 A
TITLE: SACCHAROMYCES CEREVISIAE STRAIN EXPRESSING GENE ENCODING POTASSIUM TRANSPORTER MINK

PUBN-DATE: June 27, 1995

INT-CL (IPC): C12 N 1/19; C12 N 15/09; C12 Q 1/04

APPL-NO: JP06213529
APPL-DATE: September 7, 1994

IN: KURTZ, STEPHEN E, KNICKERBOCKER, ARON M, MCCULLOUGH, JOHN R

AB: PURPOSE: To detect a yeast, recombinant DNA and inhibitor and activator of potassium channel by treating a specific modified Saccharomyces cerevisiae cell with a test compound and detecting the variation of the proliferation of the cell caused by the treatment., CONSTITUTION: A modified Saccharomyces cerevisiae cell which expresses the nucleic acid sequence of mink protein but does not express TRK1 and TRK2 is treated with a test compound and the variation of the proliferation of said cell is detected after the treatment with the test compound., COPYRIGHT: (C)1995,JPO

L5: Entry 2 of 4

File: JPAB

Jun 27, 1995

DOCUMENT-IDENTIFIER: JP 07163335 A
TITLE: SACCHAROMYCES CEREVISIAE STRAIN EXPRESSING GENE ENCODING POTASSIUM TRANSPORTER MINK

Abstract (1):
PURPOSE: To detect a yeast, recombinant DNA and inhibitor and activator of potassium channel by treating a specific modified Saccharomyces cerevisiae cell with a test compound and detecting the variation of the proliferation of the cell caused by the treatment.

3. Document ID: AU 200157898 A, WO 200151519 A2, DE 10000651 A1

L5: Entry 3 of 4

File: DWPI

Jul 24, 2001

DERWENT-ACC-NO: 2001-442137
DERWENT-WEEK: 200166

TITLE: Identifying inhibitors and activators of eukaryotic potassium

channels, for use as pharmaceuticals, comprises using yeast cells that express heterologous, but no endogenous, potassium channels

PRIORITY-DATA: 2000DE-1000651 (January 11, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200157898 A	July 24, 2001		000	C07K014/705
WO 200151519 A2	July 19, 2001	G	078	C07K014/705
DE 10000651 A1	July 26, 2001		000	C12Q001/02

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO
DESCRIPTOR		
AU 200157898A	January 5, 2001	2001AU-0057898
AU 200157898A		WO 200151519 Based on
WO 200151519A2	January 5, 2001	2001WO-EP00055
DE 10000651A1	January 11, 2000	2000DE-1000651

INT-CL (IPC): C07 K 14/705; C12 N 15/81; C12 Q 1/02
IN: LEBERER, E, LEEUW, T, RITSCHER, A

AB: NOVELTY - Identifying inhibitors or activators (A) of a eukaryotic potassium channel (KC) by applying a test compound to a mutant Saccharomyces cerevisiae cell in which:, (i) the three endogenous KC (TRK1, TRK2 and TOK1) are not expressed; but, (ii) a eukaryotic KC is expressed heterologously, where the effect of the compound on the eukaryotic KC is then determined., DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:, (1) mutant S. cerevisiae cells in which the three endogenous KC are not expressed;, (2) preparing cells of (1) by knockout destruction of the relevant genes;, (3) similar method for identifying activators of eukaryotic KC in which the cells are treated with both a test compound and a known inhibitor of KC;, (4) test kits containing the cells of (1); and, (5) preparation of pharmaceuticals using (A) identified by the new methods., ACTIVITY - None given., MECHANISM OF ACTION - Potassium channel activator or inhibitor.., USE - The method is used to identify inhibitors or activators (A) of a eukaryotic potassium channel (claimed). (A) are potentially useful as pharmaceuticals (no more details)., ADVANTAGE - The method is easily automated for parallel processing of many samples, using either different concentrations of test compounds and/or different levels of heterologous gene expression. It allows identification of compounds that inhibit human KC selectively.

L5: Entry 3 of 4

File: DWPI Jul 24, 2001

DERWENT-ACC-NO: 2001-442137
DERWENT-WEEK: 200166
COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: Identifying inhibitors and activators of eukaryotic potassium channels, for use as pharmaceuticals, comprises using yeast cells that express heterologous, but no endogenous, potassium channels

Basic Abstract Text:
NOVELTY - Identifying inhibitors or activators (A) of a eukaryotic potassium channel (KC) by applying a test compound to a mutant Saccharomyces cerevisiae cell in which:

Basic Abstract Text (1):
NOVELTY - Identifying inhibitors or activators (A) of a eukaryotic potassium channel (KC) by applying a test compound to a mutant Saccharomyces cerevisiae cell in which:

4. Document ID: AU 200140424 A, WO 200151516 A2, DE 10001230 A1

L5: Entry 4 of 4

File: DWPI Jul 24, 2001

DERWENT-ACC-NO: 2001-442135
DERWENT-WEEK: 200166
COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: Identifying inhibitors and activators of eukaryotic potassium channels, for use as therapeutic agents, comprises using a transformed yeast cell that does not express endogenous channels

PRIORITY-DATA: 2000DE-1001230 (January 13, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200140424 A	July 24, 2001		000	C07K014/005
WO 200151516 A2	July 19, 2001	G	078	C07K014/005
DE 10001230 A1	August 2, 2001		000	C07K014/005

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO
DESCRIPTOR		

AU 200140424A
January 15, 2001
2001AU-0040424
AU 200140424A
WO 200151516
Based on
WO 200151516A2
January 15, 2001
2001WO-DE00134
DE 10001230A1
January 13, 2000
2000DE-1001230

INT-CL (IPC): A61 K 38/16; C07 K 14/005
IN: GISSMANN, L, MICHEL, N, MUELLER, M, OSEN, W, ZENTGRAF, H

AB: NOVELTY - Identifying inhibitors (A) or activators (B) of a eukaryotic potassium channel by measuring the effect of a test compound on a mutant Saccharomyces cerevisiae that:, (i) does not express the endogenous potassium channels TRK1, TRK2 and TOK1; but, (ii) does express a heterologous eukaryotic potassium channel (I)., DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:, (a) mutant S. cerevisiae cell that does not express the specified endogenous channels;, (b) producing cells of (a) by knockout of the RTK1, TRK2 and TOK1 genes;, (c) identifying (B) by measuring the effect of test compounds on the mutant S. cerevisiae in presence of a known inhibitor;, (d) a test kit containing the cells of (a); and, (e) preparation of a pharmaceutical containing (A) or (B)., ACTIVITY - None given., MECHANISM OF ACTION - Potassium channel inhibitor or activator., USE - The method is used to identify substances that inhibit/activate human potassium channels selectively, potentially useful as therapeutic agents, and also to detect toxic compounds., ADVANTAGE - The method is well suited for automation and for performing many analyzes in parallel.

L5: Entry 4 of 4
File: DWPI
Jul 24, 2001

DERWENT-ACC-NO: 2001-442135
DERWENT-WEEK: 200166

TITLE: Identifying inhibitors and activators of eukaryotic potassium channels, for use as therapeutic agents, comprises using a transformed yeast cell that does not express endogenous channels

Basic Abstract Text:
NOVELTY - Identifying inhibitors (A) or activators (B) of a eukaryotic potassium channel by measuring the effect of a test compound on a mutant Saccharomyces cerevisiae that:

Basic Abstract Text (1):
NOVELTY - Identifying inhibitors (A) or activators (B) of a eukaryotic potassium channel by measuring the effect of a test compound on a mutant Saccharomyces cerevisiae that:

1. Document ID: US 20020045159 A1

L10: Entry 1 of 19

File: PGPB

Apr 18, 2002

PGPUB-DOCUMENT-NUMBER: 20020045159
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020045159 A1

TITLE: Ion channel assay methods

PUBLICATION-DATE: April 18, 2002

US-CL-CURRENT: 435/4

APPL-NO: 09/ 804457
DATE FILED: March 12, 2001

RELATED-US-APPL-DATA:
RLAN
RLFD -
RLPC
RLKC
RLAC
60217671
Jul 10, 2000
US

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. Section 119(e) to U.S. Provisional Application Ser. No. 60,217,671, entitled Instrumentation and Methods for Electrical Stimulation, filed on Jul. 10, 2000, which application is hereby incorporated by reference in its entirety. This application is also related to the following three additional U.S. patent applications, also incorporated by reference to this application in their entireties:

[0002] application Ser. No. _____, entitled ION CHANNEL ASSAY METHODS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV1.

[0003] application Ser. No. _____, entitled HIGH THROUGHPUT METHOD AND SYSTEM FOR SCREENING CANDIDATE COMPOUNDS

[0004] FOR ACTIVITY AGAINST TARGET ION CHANNELS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV2.

[0005] application Ser. No. _____, entitled MULTI-WELL PLATE AND ELECTRODE ASSEMBLIES FOR ION CHANNEL ASSAYS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV3.
IN: Maher, Michael P., Gonzalez, Jesus E. III

AB: A method of characterizing the biological activity of a candidate compound may include exposing cells to the candidate compound, and then exposing the cells to a repetitive application of electric fields so as to set the transmembrane potential to a level corresponding to a pre-selected voltage dependent state of a target ion channel.

L10: Entry 1 of 19

File: PGPB

Apr 18, 2002

DOCUMENT-IDENTIFIER: US 20020045159 A1
TITLE: Ion channel assay methods

Detail Description Paragraph (415):
[0465] The response as a function of time (FIG. 18) shows a rapid (<20 ms rise time) initial phase which decays with a time

constant of about 40 ms to a stable plateau. A small rebound potential change is also present between the spike and the plateau.
We interpret this behavior as due to the activation of endogenous voltage-dependent potassium channels (K.sub.V) that occur after the first stimulus pulse. Activation of these endogenous potassium channels would be expected to cause a reduction of transmembrane potential as potassium leaves the cell consistent with the experimental data. As electrical stimulation continues the transmembrane potential reaches a new equilibrium which is set by the balance of sodium influx into the cell and potassium efflux out of the cell. At the end of stimulation, the decay time constant of the response is about 143 ms, corresponding to a leak resistance of about 9 G.OMEGA..

2. Document ID: US 20020028480 A1

L10: Entry 2 of 19

File: PGPB

Mar 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020028480
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020028480 A1

TITLE: High throughput method and system for screening candidate compounds for activity against target ion channels

PUBLICATION-DATE: March 7, 2002

US-CL-CURRENT: 435/40; 435/173.6, 435/288.4

APPL-NO: 09/ 804580
DATE FILED: March 12, 2001

RELATED-US-APPL-DATA:
RLAN
RLFD
RLPC
RLKC
RLAC
60217671
Jul 10, 2000
US

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. Section 119(e) to U.S. Provisional Application Serial No. 60,217,671, entitled Instrumentation and Methods for Electrical Stimulation, filed on Jul. 10, 2000, which application is hereby incorporated by reference in its entirety. This application is also related to the following three additional U.S. Patent Applications, also incorporated by reference to this application in their entireties:

[0002] application Ser. No. _____,entitled ION CHANNEL ASSAY METHODS, filed Mar. 12, 2001, attorney docket AUROBIO.026A;

[0003] application Ser. No. _____, entitled ION CHANNEL ASSAY METHODS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV1.

[0004] application Ser. No. _____, entitled MULTI-WELL PLATE AND ELECTRODE ASSEMBLIES FOR ION CHANNEL ASSAYS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV3.

IN: Maher, Michael P., Gonzalez, Jesus E. III

AB: Drug candidate screening methods are applied to discover compounds with activity against ion channel targets. The method may include modulating the transmembrane potential of host cells in a plurality of sample wells with a repetitive application of electric fields so as to set the transmembrane potential to a level corresponding to a pre-selected voltage dependent state of a target ion channel.

L10: Entry 2 of 19

File: PGPB

Mar 7, 2002

DOCUMENT-IDENTIFIER: US 20020028480 A1
TITLE: High throughput method and system for screening candidate compounds for activity against target ion channels

Detail Description Paragraph (410):
[0457] The response as a function of time (FIG. 18) shows a rapid (<20 ms rise time) initial phase which decays with a time constant of about 40 ms to a stable plateau. A small rebound potential change is also present between the spike and the plateau.
We interpret this behavior as due to the activation of endogenous voltage-dependent potassium channels (K.sub.V) that occur after the first stimulus pulse. Activation of these endogenous potassium channels would be expected to cause a reduction of transmembrane potential as potassium leaves the cell consistent with the experimental data. As electrical stimulation continues the transmembrane potential reaches a new equilibrium which is set by the balance of sodium influx into the cell and potassium efflux out of the cell. At the end of stimulation, the decay time constant of the response is about 143 ms, corresponding to a leak resistance of about 9 G.OMEGA.. To determine whether this overall smaller response could be reliably used for drug discovery were conducted to determine whether the effects of TTX or tetracaine could be accurately characterized. The results shown in FIG. 19 demonstrate that the pharmacological inhibition profiles of these drugs using the present invention are consistent with the known behavior of the NAV3 sodium channel with these agents. The dose-response curve for TTX could be fitted with a Hill function with an EC.sub.50=25 nM and Hill coefficient 1.1. The dose-response curve for tetracaine could be fitted to a curve with an EC.sub.50=11 .mu.M and Hill coefficient 0.97. These results suggest that the response is caused by sodium channel activity and that pharmacological information on known and unknown compounds can be obtained using this method.

3. Document ID: US 20020025949 A1

L10: Entry 3 of 19

File: PGPB

Feb 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020025949
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020025949 A1

TITLE: Anti-epileptogenic agents

PUBLICATION-DATE: February 28, 2002

US-CL-CURRENT: 514/114; 514/381, 514/561

APPL-NO: 09/ 932676
DATE FILED: August 16, 2001

RELATED-US-APPL-DATA:
RLAN
RLFD
RLPC
RLKC
RLAC
09932676
Aug 16, 2001
GRANTED
A1
US
09041371
Mar 11, 1998
US
6306909
Mar 12, 1997
US
60041140
Feb 3, 1998
US
60073536

RELATED APPLICATIONS

[0001] This application claims benefit of priority under 35 U.S.C. 119(e) to co-pending U.S. Provisional Application Nos. 60/041,140, filed Mar. 12, 1997, and 60/073,536, filed Feb. 3, 1998. The contents of both these provisional applications is hereby incorporated by reference.

IN: Weaver, Donald E., Carran, John R.

AB: Methods and compounds useful for the inhibition of convulsive disorders, including epilepsy, are disclosed. The methods and compounds of the invention inhibit or prevent ictogenesis and epileptogenesis. Methods for preparing the compounds of the invention are also described.

L10: Entry 3 of 19
File: PGPB

Feb 28, 2002

DOCUMENT-IDENTIFIER: US 20020025949 A1
TITLE: Anti-epileptogenic agents

Summary of Invention Paragraph (23):
[0024] In another aspect, the invention provides a method for inhibiting a convulsive disorder in a subject. The method includes the step of administering to a subject in need thereof an effective amount of an agent which a) blocks sodium or calcium ion channels, or opens potassium or chloride ion channels; and b) has at least one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis and ictogenesis is inhibited in the subject. In preferred embodiments, the agent antagonizes NMDA receptors by binding to the NMDA receptors (e.g., by binding to the glycine binding site of the NMDA receptors); the agent augments GABA inhibition by decreasing glial GABA uptake; the agent is administered orally; the agent in a pharmaceutically acceptable vehicle; the agent comprises a dioxapiperazine moiety; and/or the subject is a human.

Summary of Invention Paragraph (80):
[0081] In still another aspect, the invention provides a method for inhibiting epileptogenesis and ictogenesis in a subject. The method includes the step of administering to a subject in need thereof an effective amount of an agent represented by the formula A-B, in which A is a domain having sodium or calcium ion channel blocking activity, or A has potassium or chloride channel opening activity; and B is a domain having has at least one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis is inhibited in the subject. In preferred embodiments, the domains A and B of the agent are covalently linked. In a preferred embodiment, A is a dioxapiperazine moiety.

Detail Description Paragraph (54):
[0168] In another embodiment, the invention provides a method for inhibiting both a convulsive disorder and epileptogenesis in a subject. The method includes the step of sodium or calcium ion channels, or opens potassium or chloride ion channels; and b) has at least one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis is inhibited in the subject.

4. Document ID: US 20020025573 A1

L10: Entry 4 of 19
File: PGPB

Feb 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020025573
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020025573 A1

TITLE: Multi-well plate and electrode assemblies for ion channel assays

PUBLICATION-DATE: February 28, 2002

US-CL-CURRENT: 435/287.1

APPL-NO: 09/ 804458
DATE FILED: March 12, 2001

RELATED-US-APPL-DATA:
RLAN
RLFD
RLPC
RLKC
RLAC
60217671
Jul 10, 2000
US

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. Section 119(e) to U.S. Provisional Application Serial No. 60, 217, 671, entitled Instrumentation and Methods for Electrical Stimulation, filed on Jul. 10, 2000, which application is hereby incorporated by reference in its entirety. This application is also related to the following three additional U.S. patent applications, also incorporated by reference to this application in their entireties:

[0002] Application Ser. No. _____, entitled ION CHANNEL ASSAY METHODS, filed Mar. 12, 2001, attorney docket AUROBIO.026A;

[0003] Application Ser. No. _____, entitled ION CHANNEL ASSAY METHODS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV1.

[0004] Application Ser. No. _____, entitled HIGH THROUGHPUT METHOD AND SYSTEM FOR SCREENING CANDIDATE COMPOUNDS FOR ACTIVITY AGAINST TARGET ION CHANNELS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV2.

IN: Maher, Michael P., Gonzalez, Jesus E. III

AB: Plate and electrode assemblies include configurations allowing for relatively uniform electric field
production. The electrodes may comprise strips of conductive material plated onto the bottom surface of sample wells
or they may comprise plate electrodes extending down into the well. In some embodiments, the electric field strength
varies by less than about 10% from a mean field intensity over at least about 20% of the surface area of the bottom
surface of a sample well.

L10: Entry 4 of 19 File: PGPB

Feb 28, 2002

DOCUMENT-IDENTIFIER: US 20020025573 A1
TITLE: Multi-well plate and electrode assemblies for ion channel assays

Detail Description Paragraph (416):
[0465] The response as a function of time (FIG. 18) shows a rapid (<20 ms rise time) initial phase which decays with a time
constant of about 40 ms to a stable plateau. A small rebound potential change is also present between the spike and the plateau.
We interpret this behavior as due to the activation of endogenous voltage-dependent potassium channels (K.sub.v) that occur after
the first stimulus pulse. Activation of these endogenous potassium channels would be expected to cause a reduction of
transmembrane potential as potassium leaves the cell consistent with the experimental data. As electrical stimulation continues
the transmembrane potential reaches a new equilibrium which is set by the balance of sodium influx into the cell and potassium
efflux out of the cell. At the end of stimulation, the decay time constant of the response is about 143 ms, corresponding to a
leak resistance of about 9 G.OMEGA..

5. Document ID: US 20020025568 A1

L10: Entry 5 of 19 File: PGPB

Feb 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020025568
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020025568 A1

TITLE: Ion channel assay methods

PUBLICATION-DATE: February 28, 2002

US-CL-CURRENT: 435/173.6

APPL-NO: 09/ 804480
DATE FILED: March 12, 2001

RELATED-US-APPL-DATA:
RLAN
RLFD
RLPC
RLKC
RLAC
60217671
Jul 10, 2000
US

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. Section 119(e) to U.S. Provisional Application Serial No. 60,217,671,
entitled Instrumentation and Methods for Electrical Stimulation, filed on Jul. 10, 2000, which application is hereby incorporated
by reference in its entirety. This application is also related to the following three additional U.S. patent applications, also
incorporated by reference to this application in their entireties:

[0002] application Ser. No. _____, entitled ION CHANNEL ASSAY METHODS, filed Mar. 12, 2001, attorney docket AUROBIO.026A;

[0003] application Ser. No. _____, entitled HIGH THROUGHPUT METHOD AND SYSTEM FOR SCREENING CANDIDATE COMPOUNDS FOR ACTIVITY
AGAINST TARGET ION CHANNELS, filed Mar. 12, 2001, attorney docket AUROBIO.026DV2.

[0004] application Ser. No. _____, entitled MULTI-WELL PLATE AND ELECTRODE ASSEMBLIES FOR ION CHANNEL ASSAYS, filed Mar. 12,
2001, attorney docket AUROBIO.026DV3.
IN: Maher, Michael P., Gonzalez, Jesus E. III

AB: A method of characterizing the biological activity of a candidate compound may include exposing cells to
the candidate compound, and then exposing the cells to a repetitive application of electric fields so as to set the
transmembrane potential to a level corresponding to a pre-selected voltage dependent state of a target ion channel.

L10: Entry 5 of 19 File: PGPB

Feb 28, 2002

DOCUMENT-IDENTIFIER: US 20020025568 A1
TITLE: Ion channel assay methods

Detail Description Paragraph (415):
[0465] The response as a function of time (FIG. 18) shows a rapid (<20 ms rise time) initial phase which decays with a time
constant of about 40 ms to a stable plateau. A small rebound potential change is also present between the spike and the plateau.
We interpret this behavior as due to the activation of endogenous voltage-dependent potassium channels (K.sub.v) that occur after
the first stimulus pulse. Activation of these endogenous potassium channels would be expected to cause a reduction of
transmembrane potential as potassium leaves the cell consistent with the experimental data. As electrical stimulation continues
the transmembrane potential reaches a new equilibrium which is set by the balance of sodium influx into the cell and potassium
efflux out of the cell. At the end of stimulation, the decay time constant of the response is about 143 ms, corresponding to a
leak resistance of about 9 G.OMEGA..

6. Document ID: US 20010031243 A1

L10: Entry 6 of 19 File: PGPB

Oct 18, 2001

PGPUB-DOCUMENT-NUMBER: 20010031243

PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010031243 A1

TITLE: Novel methods of ultrasound treatment using gas or gaseous precursor-filled compositions

PUBLICATION-DATE: October 18, 2001

US-CL-CURRENT: 424/9.51; 424/9.52, 604/20

APPL-NO: 09/ 813484
DATE FILED: March 21, 2001

RELATED-US-APPL-DATA:

RLAN	RLFD	RLPC	RLKC	RLAC
09813484	Mar 21, 2001	PENDING	A1	US
08929847	Sep 15, 1997			

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of copending U.S. patent application Ser. No. 081929,847, filed Sep. 15, 1997, which is incorporated herein by reference in its entirety.

IN: Unger, Evan C.

AB: The present invention describes, among other things, the surprising discovery that gaseous precursor filled compositions are profoundly more effective as acoustically active contrast agents when they are thermally preactivated to temperatures at or above the boiling point of the instilled gaseous precursor prior to their in vivo administration to a patient. Further optimization of contrast enhancement is achieved by administering the gaseous precursor filled compositions to a patient as an infusion. Enhanced effectiveness is also achieved for ultrasound mediated targeting and drug delivery.

L10: Entry 6 of 19

File: PGPB

Oct 18, 2001

DOCUMENT-IDENTIFIER: US 20010031243 A1
TITLE: Novel methods of ultrasound treatment using gas or gaseous precursor-filled compositions

Detail Description Paragraph (304):
[0304] A wide variety of targeting ligands may be selected for targeting myocardial cells. Exemplary targeting ligands include, for example, anticardiomyosin antibody, which may comprise polyclonal antibody, Fab'2 fragments, or be of human origin, animal origin, for example, mouse origin, or of chimeric origin. Additional targeting ligands include dipyridamole; digitalis; nifedipine; apolipoprotein; low density lipoproteins (LDL), including .alpha.-LDL, vLDL and methyl LDL; ryanodine; endothelin; complement receptor type 1; IgG Fc; beta 1-adrenergic; dihydropyridine; adenosine; mineralocorticoid; nicotinic acetylcholine and muscarinic acetylcholine; antibodies to the human alpha 1A-adrenergic receptor; bioactive agents, such as drugs, including the alpha 1-antagonist prazosin; antibodies to the anti-beta-receptor; drugs which bind to the anti-beta-receptor; anti-cardiac RyR antibodies; endothelin-1, which is an endothelial cell-derived vasoconstrictor peptide that exerts a potent positive inotropic effect on cardiac tissue (endothelin-1 binds to cardiac sarcolemmal vesicles); monoclonal antibodies which may be generated to the T-cell receptor .alpha.-.beta.receptor and thereby employed to generate targeting ligands; the complement inhibitor sCR1; drugs, peptides or antibodies which are generated to the dihydropyridine receptor; monoclonal antibodies directed towards the anti-interleukin-2 receptor may be used as targeting ligands to direct the present compositions to areas of myocardial tissue which express this receptor and which may be up-regulated in conditions of inflammation; cyclosporine for directing similarly the compositions to areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes of cardiac myocytes and cardiac endothelial cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing peptide; atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell origin and is structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and antimitogenic activity; vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) and comprises 27 amino acids; thrombin; endothelium-derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1); competitive inhibitors to EDRF, including, for example, NG-monomethyl-L-arginine (L-NMMA); potassium channel antagonists, such as charybdotoxin and glibenclamide; antiheart antibodies, which may be identified in patients with idiopathic dilated cardiomyopathy but which preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator, the branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be referred to as BQ-123; and antibodies to the angiotensin II receptor.

7. Document ID: US 6358699 B1

L10: Entry 7 of 19

File: USPT

Mar 19, 2002

US-PAT-NO: 6358699
DOCUMENT-IDENTIFIER: US 6358699 B1

TITLE: Assay for asymmetrical NG, NG dimethyl-l-arginine

DATE-ISSUED: March 19, 2002

US-CL-CURRENT: 435/18; 435/23, 435/24, 435/962, 435/968, 435/975

APPL-NO: 9/ 495553
DATE FILED: February 1, 2000

PARENT-CASE:

This is a continuation-in-part application of copending prior application Ser. No. 60/118,838, filed on Feb. 5, 1999.

IN: Balint; Robert F., Mutz; Mitchell Wayne, Cooke; John P.

AB: ADMA is determined in a physiological sample by first removing interfering components: proteins by precipitation; and amines and citrulline, by means of a cation exchange column, followed by enzymatic hydrolysis of the ADMA to citrulline with DDAH. The citrulline is then determined spectrophotometrically.

L10: Entry 7 of 19

File: USPT

Mar 19, 2002

DOCUMENT-IDENTIFIER: US 6358699 B1
TITLE: Assay for asymmetrical NG, NG dimethyl-l-arginine

Detailed Description Paragraph Type 0 (17):
Cooke J P, Rossitch E, Andon N, Loscalzo J, Dzau V J: Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. J Clin Invest 1991a;88:1663-1671

8. Document ID: US 6350431 B1

L10: Entry 8 of 19
File: USPT

Feb 26, 2002

US-PAT-NO: 6350431
DOCUMENT-IDENTIFIER: US 6350431 B1

TITLE: Compounds

DATE-ISSUED: February 26, 2002

US-CL-CURRENT: 424/9.6; 548/223, 549/402, 549/427, 549/455

APPL-NO: 9/ 429347
DATE FILED: October 28, 1999

PARENT-CASE:

This application is a continuation of international application No. PCT/GB98/01244 filed Apr. 29, 1998 (of which the entire disclosure of the pending, prior application is hereby incorporated by reference), which itself is a CIP of U.S. application Ser. No. 08/848,586 filed Apr. 29, 1997, now abandoned, and a CIP of U.S. application Ser. No. 09/035,285 filed Mar. 5, 1998, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9727124	December 22, 1997

IN: Snow; Robert Allen, Henrichs; Paul Mark, Delecki; Daniel Joseph, Sanderson; William Anthony, Desai; Vinay Chandrakant, Bacon; Edward, Hollister; Kenneth Robert, Hohenschuh; Eric Paul

AB: This invention provides a physiologically tolerable light imaging contrast agent compound having a molecular weight in the range 500 to 5000000 and containing at least two chromophores having delocalized electron systems as well as at least one polyalkylene oxide (PAO) moiety having a molecular weight in the range 60 to 100000.

L10: Entry 8 of 19
File: USPT

Feb 26, 2002

DOCUMENT-IDENTIFIER: US 6350431 B1
TITLE: Compounds

Brief Summary Paragraph Right (134):
A wide variety of targeting vectors may be selected for targeting myocardial cells. Exemplary targeting vectors include, for example, anticardiomyosin antibody, which may comprise polyclonal antibody, Fab'2 fragments, or be of human origin, animal origin, for example, mouse origin, or of chimeric origin. Additional targeting vectors include dipyridamole; digitalis; nifedipine; apolipoprotein; low density lipoproteins (LDL), including alpha-LDL, vLDL and methyl LDL; ryanodine; endothelin; complement receptor type 1; IgG Fc; beta 1-adrenergic; dihydropyidine; adenosine; mineralocorticoid; nicotinic acetylcholine and muscarinic acetylcholine; antibodies to the human alpha 1A-adrenergic receptor; bioactive agents, such as drugs, including the alpha 1-antagonist prazosin; antibodies to the anti-beta-receptor; drugs which bind to the anti-beta-receptor, anticardiac RyR antibodies; endothelin-1, which is an endothelial cell-derived vasoconstrictor peptide that exerts a potent positive inotropic effect on cardiac tissue (endothelin-1 binds to cardiac sarcolemmal vesicles); monoclonal antibodies which may be generated to the T cell receptor alpha-beta receptor and thereby employed to generate targeting vectors; the complement inhibitor sCR1; drugs, peptides or antibodies which are generated to the dihydropyridine receptor; monoclonal antibodies directed towards the antiinterleukin 2 receptor may be used as targeting vectors to direct the present chromophore polymer compounds and compositions to areas of myocardial tissue which express this receptor and which may be up-regulated in conditions of inflammation; cyclosporine for similarly directing the compositions to areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes of cardiac myocytes and cardiac endothelial cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing peptide; atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell origin and is structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and antimitogenic activity; vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type nathuretic peptide (CNP) and comprises 27 amino acids; thrombin; endothelium derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1); competitive inhibitors to EDRF, including, for example, NG-monomethyl-L-arginine (L-NNMA); potassium channel antagonists, such as charybdotoxin and glibenclanmide; antiheart antibodies, which can be identified in patients with idiopathic dilated cardiomyopathy but which preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator, the branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be referred to as BQ-123; and antibodies to the angiotensin II receptor.

9. Document ID: US 6306909 B1

L10: Entry 9 of 19
File: USPT

Oct 23, 2001

US-PAT-NO: 6306909
DOCUMENT-IDENTIFIER: US 6306909 B1

TITLE: Anti-epileptogenic agents

DATE-ISSUED: October 23, 2001

US-CL-CURRENT: 514/561; 514/567, 562/507, 562/576

APPL-NO: 9/ 041371
DATE FILED: March 11, 1998

PARENT-CASE:

RELATED APPLICATIONS
This application claims benefit of priority under 35 U.S.C. 119(e) to co-pending U.S. Provisional Application Nos. 60/041,140, filed Mar. 12, 1997, and 60/073,536, filed Feb. 3, 1998. The contents of both these provisional applications are hereby incorporated by reference.

IN: Weaver; Donald E., Milne; Paul H., Tan; Christopher Y. K., Carran; John R.

AB: Methods and compounds useful for the inhibition of convulsive disorders, including epilepsy, are disclosed. The methods and compounds of the invention inhibit or prevent ictogenesis and epileptogenesis. Methods for preparing the compounds of the invention are also described.

L10: Entry 9 of 19 File: USPT

Oct 23, 2001

DOCUMENT-IDENTIFIER: US 6306909 B1
TITLE: Anti-epileptogenic agents

Brief Summary Paragraph Right (14):
In another aspect, the invention provides a method for inhibiting a convulsive disorder in a subject. The method includes the step of administering to a subject in need thereof an effective amount of an agent which a) blocks sodium or calcium ion channels, or opens potassium or chloride ion channels; andb) has at Icast one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis and ictogenesis is inhibited in the subject. In preferred embodiments, the agent antagonizes NMDA receptors by binding to the NMDA receptors (e.g., by binding to the glycine binding site of the NMDA receptors); the agent augments GABA inhibition by decreasing glial GABA uptake; the agent is administered orally; the agent in a pharmaceutically acceptable vehicle; the agent comprises a dioxapiperazine moiety; and/or the subject is a human.

Brief Summary Paragraph Right (21):
In still another aspect, the invention provides a method for inhibiting epileptogenesis and ictogenesis in a subject. The method includes the step of administering to a subject in need thereof an effective amount of an agent represented by the formula A--B, in which A is a domain having sodium or calcium ion channel blocking activity, or A has potassium or chloride channel opening activity; and B is a domain having has at least one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis is inhibited in the subject. In preferred embodiments, the domains A and B of the agent are covalently linked. In a preferred embodiment, A is a dioxapiperazine moiety.

Detailed Description Paragraph Right (47):
In another embodiment, the invention provides a method for inhibiting both a convulsive disorder and epileptogenesis in a subject. The method includes the step of administering to a subject in need thereof an effective amount of an agent which a) blocks sodium or calcium ion channels, or opens potassium or chloride ion channels; and b) has at least one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis is inhibited in the subject.

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10. Document ID: US 6231834 B1

L10: Entry 10 of 19 File: USPT

May 15, 2001

US-PAT-NO: 6231834
DOCUMENT-IDENTIFIER: US 6231834 B1

TITLE: Methods for ultrasound imaging involving the use of a contrast agent and multiple images and processing of same

DATE-ISSUED: May 15, 2001

US-CL-CURRENT: 424/9.51; 424/9.52, 600/431

APPL-NO: 8/ 982829
DATE FILED: December 2, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of U.S. application Ser. No. 08/932,273, filed Sep. 17, 1997, now allowed, which in turn is a continuation-in-part of application Ser. No. 08/666,129, filed on Jun. 19, 1996 now U.S. Pat. No. 6,033,645, issued, Mar. 7, 2000 and U.S. application Ser. No. 08/660,032, filed Jun. 6, 1996, now abandoned, the latter of which is a continuation-in-part of U.S. application Ser. No. 08/640,464, filed May 1, 1996, now abandoned, which is a continuation-in-part of U.S. application Ser. No.08/497,684, filed Jun. 7, 1995, now abandoned. The disclosures of each of the foregoing applications are hereby incorporated herein by reference, in their entireties.

IN: Unger; Evan C., Fritz; Thomas A., Gertz; Edward W.

AB: Improved methods for providing an image of an internal region of a patient. Embodiments of the invention involve the administration to the patient of a contrast agent which comprises, in an aqueous carrier, a lipid, protein, polymer or surfactant, and a gas. The patient is scanned using ultrasound imaging to obtain a visible image of the region. In embodiments of the invention, the scanning step may comprise applying a first quantity of ultrasound energy to the patient to provide a first image, followed by the application substantially immediately thereafter of a second quantity of ultrasound energy to provide a second image. The first and second images are then processed. The methods are particularly useful for obtaining on-line images of the cardiovascular region which may be employed, for example, to diagnose the presence of diseased tissue in the cardiovascular region of a patient.

L10: Entry 10 of 19 File: USPT

May 15, 2001

DOCUMENT-IDENTIFIER: US 6231834 B1
TITLE: Methods for ultrasound imaging involving the use of a contrast agent and multiple images and processing of same

Detailed Description Paragraph Right (126):
A wide variety of targeting ligands may be selected for targeting myocardial cells. Exemplary targeting ligands include, for example, anticardiomyosin antibody, which may comprise polyclonal

antibody, Fab'2 fragments, or be of human origin, animal origin, for example, mouse origin, or of chimeric origin. Additional targeting ligands include dipyridamole; digitalis; nifedipine; apolipoprotein; low density lipoproteins (LDL), including .alpha.-LDL, vLDL and methyl LDL; ryanodine; endothelin; complement receptor type 1; IgG Fc; beta 1-adrenergic; dihydropyridine; adenosine; mineralocorticoid; nicotinic acetylcholine and muscarinic acetylcholine; antibodies to the human alpha 1A-adrenergic receptor; bioactive agents, such as drugs, including the alpha 1-antagonist prazosin; antibodies to the anti-beta-receptor; drugs which bind to the anti-beta-receptor; anti-cardiac RyR antibodies; endothelin-1, which is an endothelial cell-derived vasoconstrictor peptide that exerts a potent positive inotropic effect on cardiac tissue (endothelin-1 binds to cardiac sarcolemmal vesicles); monoclonal antibodies which may be generated to the T cell receptor alpha-beta receptor and thereby employed to generate targeting ligands; the complement inhibitor sCR1; drugs, peptides or antibodies which are generated to the dihydropyridine receptor; monoclonal antibodies directed towards the antiinterleukin 2 receptor may be used as targeting ligands to direct the present compositions to areas of myocardial tissue which express this receptor and which may be up-regulated in conditions of inflammation; cyclosporine for directing similarly the compositions to areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes of cardiac myocytes and cardiac endothelial cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing peptide; atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell origin and is structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and antimitogenic activity; vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) and comprises 27 amino acids; thrombin; endothelium-derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1); competitive inhibitors to EDRF, including, for example, NG-monomethyl-L-arginine (L-NMMA); potassium channel antagonists, such as charybdotoxin and glibenclamide; antiheart antibodies, which may be identified in patients with idiopathic dilated cardiomyopathy but which preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator, the branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be referred to as BQ-123; and antibodies to the angiotensin II receptor.

11. Document ID: US 6139819 A

L10: Entry 11 of 19 File: USPT

Oct 31, 2000

US-PAT-NO: 6139819
DOCUMENT-IDENTIFIER: US 6139819 A

TITLE: Targeted contrast agents for diagnostic and therapeutic use

DATE-ISSUED: October 31, 2000

US-CL-CURRENT: 424/9.52; 424/450, 424/9.51

APPL-NO: 8/ 932273
DATE FILED: September 17, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of U.S. application Ser. No. 08/660,032, filed Jun. 6, 1996, now abandoned which in turn is a continuation-in-part of U.S. application Ser. No. 08/640,464, filed May 1, 1996, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 08/497,684, filed Jun. 7, 1995, now abandoned. This application is also a continuation-in-part of U.S. application Ser. No. 08/666,129, filed Jun. 19, 1996, now U.S. Pat. No. 6,033,645. The disclosures of each of the foregoing applications are hereby incorporated herein by reference, in their entireties.

IN: Unger; Evan C., Fritz; Thomas A., Gertz; Edward W.

AB: Novel contrast agents which may be used for diagnostic and therapeutic use. The compositions may comprise a lipid, a protein, polymer and/or surfactant, and a gas, in combination with a targeting ligand. In preferred embodiments, the targeting ligand targets coagula, including emboli and/or thrombi, particularly in patients suffering from an arrhythmic disorder. The contrast media can be used in conjunction with diagnostic imaging, such as ultrasound, as well as therapeutic applications, such as therapeutic ultrasound.

L10: Entry 11 of 19 File: USPT

Oct 31, 2000

DOCUMENT-IDENTIFIER: US 6139819 A
TITLE: Targeted contrast agents for diagnostic and therapeutic use

Detailed Description Paragraph Right (133):
directing similarly the compositions to areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes of cardiac myocytes and cardiac endothelial cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing peptide; atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell origin and is structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and antimitogenic activity; vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) and comprises 27 amino acids; thrombin; endothelium-derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1); competitive inhibitors to EDRF, including, for example, NG-monomethyl-L-arginine (L-NMMA); potassium channel antagonists, such as charybdotoxin and glibenclamide; antiheart antibodies, which may be identified in patients with idiopathic dilated cardiomyopathy but which preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator, the branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be referred to as BQ-123; and antibodies to the angiotensin II receptor.

12. Document ID: US 6123923 A

L10: Entry 12 of 19 File: USPT

Sep 26, 2000

US-PAT-NO: 6123923
DOCUMENT-IDENTIFIER: US 6123923 A

TITLE: Optoacoustic contrast agents and methods for their use

DATE-ISSUED: September 26, 2000

US-CL-CURRENT: 424/9.52; 424/450, 424/9.1, 424/9.2, 424/9.3, 424/9.6, 514/410

APPL-NO: 8/ 993165
DATE FILED: December 18, 1997
IN: Unger; Evan C., Wu; Yunqiu

AB: The present invention generally relates to optoacoustic contrast agents and methods of diagnostic and
therapeutic imaging using optoacoustic contrast agents.

L10: Entry 12 of 19

File: USPT

Sep 26, 2000

DOCUMENT-IDENTIFIER: US 6123923 A
TITLE: Optoacoustic contrast agents and methods for their use

Detailed Description Paragraph Right (183):
A wide variety of targeting ligands may be selected for targeting myocardial cells. Suitable targeting ligands include, for
example, anticardiomyosin antibody, which may comprise polyclonal antibody, Fab'2 fragments, or be of human origin, animal origin,
for example, mouse origin, or of chimeric origin. Additional targeting ligands include dipyridamole; digitalis; nifedipine;
apolipoprotein; low density lipoproteins (LDL), including .alpha.-LDL, vLDI, and methyl LDL; ryanodine; endothelin; complement
receptor type 1; IgG Fc; beta 1-adrenergic; dihydropyridine; adenosine; mineralocorticoid; nicotinic acetylcholine and muscarinic
acetylcholine; antibodies to the human alpha 1A-adrenergic receptor; pharmaceuticals, such as drugs, including the alpha
1-antagonist prazosin; antibodies to the anti-beta-receptor; drugs which bind to the anti-beta-receptor; anti-cardiac RyR
antibodies; endothelin-1, which is an endothelial cell-derived vasoconstrictor peptide that exerts a potent positive inotropic
effect on cardiac tissue (endothelin-1 binds to cardiac sarcolemmal vesicles); monoclonal antibodies which may be generated to the
T-cell receptor (x -P receptor and thereby employed to generate targeting ligands; the complement inhibitor sCRI; drugs, peptides
or antibodies which are generated to the dihydropyridine receptor; monoclonal antibodies directed towards the anti-interleukin-2
receptor may be used as targeting ligands to direct the present compositions to areas of myocardial tissue which express this
receptor and which may be up-regulated in conditions of inflammation; cyclosporine for directing similarly the compositions to
areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes of cardiac
myocytes and cardiac endothelial cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing peptide; atrial
natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell origin and is
structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and antimitogenic activity;
vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) and comprises
27 amino acids; thrombin; endothelium-derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1); competitive inhibitors to
EDRF, including, for example, NG-monomethyl-L-arginine (L-NMMA); potassium channel antagonists, such as charybdotoxin and
glibenclamide; antiheart antibodies, which may be identified in patients with idiopathic dilated cardiomyopathy but which
preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator, the
branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be
referred to as BQ-123; and antibodies to the angiotensin II receptor.

13. Document ID: US 6120751 A

L10: Entry 13 of 19

File: USPT

Sep 19, 2000

US-PAT-NO: 6120751
DOCUMENT-IDENTIFIER: US 6120751 A

TITLE: Charged lipids and uses for the same

DATE-ISSUED: September 19, 2000

US-CL-CURRENT: 424/9.51; 264/4, 264/4.1, 424/450, 424/502, 424/9.52, 428/402.2

APPL-NO: 8/ 925353
DATE FILED: September 8, 1997

PARENT-CASE:

RELATED APPLICATIONS
This is a continuation-in-part of U.S. application Ser. No. 08/823,791, filed Mar. 21, 1997; a continuation-in-part of U.S.
application Ser. No. 08/851,780 filed May 6, 1997; a continuation-in-part of U.S. application Ser. No. 08/877,826 filed Jun. 18,
1997; and a continuation-in-part of U.S. application Ser. No. 08/887,215 filed Jul. 2, 1997, the disclosures of each of which are
hereby incorporated by reference hirein in their entirety.
IN: Unger; Evan C.

AB: The present invention is directed to charged lipids, compositions comprising charged lipids, and the use
of these compositions in drug delivery, targeted drug delivery, therapeutic imaging and diagnostic imaging, as well
as their use as contrast agents.

L10: Entry 13 of 19

File: USPT

Sep 19, 2000

DOCUMENT-IDENTIFIER: US 6120751 A
TITLE: Charged lipids and uses for the same

Detailed Description Paragraph Right (187):
A wide variety of targeting ligands may be selected for targeting myocardial cells. Exemplary targeting ligands include, for
example, anticardiomyosin antibody, which may comprise polyclonal antibody, Fab'2 fragments, or be of human origin, anima origin,
for example, mouse origin, or of chimeric origin. Additional targeting ligands include dipyridamole; digitalis; nifedipine;
apolipoprotein; low density lipoproteins (LDL), including .alpha.-LDL, vLDL and methyl LDL; ryanodine; endothelin; complement
receptor type 1; IgG Fc; beta 1-adrenergic; dihydropyridine; adenosine; mineralocorticoid; nicotinic acetylcholine and muscarinic
acetylcholine; antibodies to the human alpha 1A-adrenergic receptor; bioactive agents, such as drugs, including the alpha
1-antagonist prazosin; antibodies to the anti-beta-receptor; drugs which bind to the anti-beta-receptor; anti-cardiac RyR
antibodies; endothelin-1, which is an endothelial cell-derived vasoconstrictor peptide that exerts a potent positive inotropic
effect on cardiac tissue (endothelin-1 binds to cardiac sarcolemmal vesicles); monoclonal antibodies which may be generated to the
T-cell receptor .alpha.-.beta. receptor and thereby employed to generate targeting ligands; the complement inhibitor sCR1; drugs,
peptides or antibodies which are generated to the dihydropyridine receptor; monoclonal antibodies directed towards the

anti-interleukin-2 receptor may be used as targeting ligands to direct the present compositions to areas of myocardial tissue which express this receptor and which may be up-regulated in conditions of inflammation; cyclosporine for directing similarly the compositions to areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes of cardiac myocytes and cardiac endotheli,al cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing peptide; atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell origin and is structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and antimitogenic activity; vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) and comprises 27 amino acids; thrombin; endothelium-derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1); competitive inhibitors to EDRF, including, for example, NG-monomethyl-L-arginine (L-NMMA); potassium channel antagonists, such as charybdotoxin and glibenclamide; antiheart antibodies, which may be identified in patients with idiopathic dilated cardiomyopathy but which preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator, the branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be referred to as BQ-123; and antibodies to the angiotensin II receptor.

14. Document ID: US 6090800 A

L10: Entry 14 of 19 File: USPT Jul 18, 2000

US-PAT-NO: 6090800
DOCUMENT-IDENTIFIER: US 6090800 A

TITLE: Lipid soluble steroid prodrugs

DATE-ISSUED: July 18, 2000

US-CL-CURRENT: 514/180; 552/574

APPL-NO: 8/ 851780
DATE FILED: May 6, 1997
IN: Unger; Evan C., Shen; DeKang

AB: The present invention is directed to novel lipid soluble steroid prodrugs compositions comprising steroid prodrugs, and uses of the same.

L10: Entry 14 of 19 File: USPT Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090800 A
TITLE: Lipid soluble steroid prodrugs

Brief Summary Paragraph Right (180):
A wide variety of targeting ligands may be selected for targeting myocardial cells. Exemplary targeting ligands include, for example, anticardiomyosin antibody, which may comprise polyclonal antibody, Fab'2 fragments, or be of human origin, animal origin, for example, mouse origin, or of chimeric origin. Additional targeting ligands include dipyridamole; digitalis; nifedipine; apolipoprotein; low density lipoproteins (LDL), including .alpha.-LDL, vLDL and methyl LDL; ryanodine; endothelin, complement receptor type 1; IgG Fc; beta 1-adrenergic; dihydropyridine; adenosine; mineralocorticoid; nicotinic acetylcholine and muscarinic acetylcholine; antibodies to the human alpha 1A-adrenergic receptor; bioactive agents, such as drugs, including the alpha 1-antagonist prazosin; antibodies to the anti-beta-receptor; drugs which bind to the anti-beta-receptor; anti-cardiac RyR antibodies; endothelin-1, which is an endothelial cell-derived vasoconstrictor peptide that exerts a potent positive inotropic effect on cardiac tissue (endothelin-1 binds to cardiac sarcolemmal vesicles); monoclonal antibodies which may be generated to the T-cell receptor .alpha.-.beta. receptor and thereby employed to generate targeting ligands; the complement inhibitor sCR1; drugs, peptides or antibodies which are generated to the dihydropyridine receptor; monoclonal antibodies directed towards the anti-interleukin-2 receptor may be used as targeting ligands to direct the present compositions to areas of myocardial tissue which express this receptor and which may be up-regulated in conditions of inflammation; cyclosporine for directing similarly the compositions to areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes of cardiac myocytes and cardiac endothelial cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing peptide; atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell origin and is structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and antimitogenic activity; vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) and comprises 27 amino acids; thrombin; endothelium-derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1); competitive inhibitors to EDRF, including, for example, NG-monomethyl-L-arginine (L--NMMA); potassium channel antagonists, such as charybdotoxin and glibenclamide; antiheart antibodies, which may be identified in patients with idiopathic dilated cardiomyopathy but which preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator, the branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be referred to as BQ-123; and antibodies to the angiotensin II receptor.

15. Document ID: US 6046046 A

L10: Entry 15 of 19 File: USPT Apr 4, 2000

US-PAT-NO: 6046046
DOCUMENT-IDENTIFIER: US 6046046 A

TITLE: Compositions, methods and devices for maintaining an organ

DATE-ISSUED: April 4, 2000

US-CL-CURRENT: 435/284.1; 435/286.5, 435/286.6

APPL-NO: 9/ 054698
DATE FILED: April 3, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED U.S. APPLICATION
The present application is a continuation-in-part of U.S. Ser. No. 08/936,062, filed Sep. 23, 1997, currently pending.
IN: Hassanein; Waleed H.

AB: Compositions, methods, systems/devices and media are provided for maintaining a harvested organ in a
functioning and viable state prior to implantation. The organ perfusion apparatus includes a preservation chamber
for storing the organ during the preservation period. A perfusion circuit is provided having a first line for
providing an oxygenated fluid to the organ, and a second line for carrying depleted fluid away from the organ. The
perfusion apparatus also includes a device operably associated with the perfusion circuit for maintaining the organ
at a substantially normothermic temperature.

L10: Entry 15 of 19 File: USPT

Apr 4, 2000

DOCUMENT-IDENTIFIER: US 6046046 A
TITLE: Compositions, methods and devices for maintaining an organ

Detailed Description Paragraph Right (87):
Examples of constituents which may be employed in the fluid media and/or preservation solution of the present invention include,
without limitation: carbohydrates (glucose, dextrose); electrolytes (sodium, potassium, bicarbonates, calcium, magnesium);
antibiotics and antimicrobials (gram negative and gram positive, e.g., penicillin at 250,000 to 1,000,000 units, preferably
250,000 units); hormones (insulin, epinephrin); endogenous metabolites or precursors of endogenous metabolites (adenosine,
L-Arginine); fatty acids (saturated and unsaturated, short chain and long chain); and conventional pharmaceutically-active agents
(such as heparin, nitroglycerin, ACE inhibitors, beta-blockers, calcium channel blockers, cytoprotective agents, antioxidants,
complements, anti-complements, immunosuppressive agents, nonsteroidal anti-inflammatories, steroids, vitamins, enzymes,
co-enzymes, and the like); and other materials conventionally employed for intravenous administration or direct injection to
assist in delivery, bioavailability, or stability of the solution. Other constituents can also be used (as will be appreciated by
the skilled artisan) that control pH, stabilize the solution, control viscosity, etc.

16. Document ID: US 6028066 A

L10: Entry 16 of 19 File: USPT

Feb 22, 2000

US-PAT-NO: 6028066
DOCUMENT-IDENTIFIER: US 6028066 A

TITLE: Prodrugs comprising fluorinated amphiphiles

DATE-ISSUED: February 22, 2000

US-CL-CURRENT: 514/180; 514/169, 552/507

APPL-NO: 8/ 887215
DATE FILED: July 2, 1997

PARENT-CASE:

RELATED APPLICATIONS
This is a continuation-in-part of U.S. application Ser. No. 08/851,780, filed May 6, 1997, the disclosure of which is hereby
incorporated by reference herein in its entirety.

IN: Unger; Evan C.

AB: The present invention describes, inter alia, novel prodrugs comprising fluorinated amphiphiles,
compositions comprising the novel prodrugs, and methods of use of the prodrugs and compositions.

L10: Entry 16 of 19 File: USPT

Feb 22, 2000

DOCUMENT-IDENTIFIER: US 6028066 A
TITLE: Prodrugs comprising fluorinated amphiphiles

Brief Summary Paragraph Right (205):
A wide variety of targeting ligands may be selected for targeting myocardial cells. Exemplary targeting ligands include, for
example, anticardiomyosin antibody, which may comprise polyclonal antibody, Fab'2 fragments, or be of human origin, animal origin,
for example, mouse origin, or of chimeric origin. Additional targeting ligands include dipyridamole; digitalis; nifedipine;
apolipoprotein; low density lipoproteins (LDL), including .alpha.-LDL, vLDL and methyl LDL; ryanodine; endothelin; complement
receptor type 1; IgG Fc; beta 1-adrenergic; dihydropyridine; adenosine; mineralocorticoid; nicotinic acetylcholine and muscarinic
acetylcholine; antibodies to the human alpha 1A-adrenergic receptor; bioactive agents, such as drugs, including the alpha
1-antagonist prazosin; antibodies to the anti-beta-receptor; drugs which bind to the anti-beta-receptor; anti-cardiac RyR
antibodies; endothelin-1, which is an endothelial cell-derived vasoconstrictor peptide that exerts a potent positive inotropic
effect on cardiac tissue (endothelin-1 binds to cardiac sarcolemmal vesicles); monoclonal antibodies which may be generated to the
T-cell receptor .alpha.-.beta. receptor and thereby employed to generate targeting ligands; the complement inhibitor sCR1; drugs,
peptides or antibodies which are generated to the dihydropyridine receptor; monoclonal antibodies directed towards the
anti-interleukin-2 receptor may be used as targeting ligands to direct the present compositions to areas of myocardial tissue
which express this receptor and which may be up-regulated in conditions of inflammation; cyclosporine for directing similarly the
compositions to areas of inflamed myocardial tissue; methylisobutyl isonitrile; lectins which bind to specific sugars on membranes
of cardiac myocytes and cardiac endothelial cells; adrenomedullin (ADM), which is an endogenous hypotensive and vasorelaxing
peptide; atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP), which is a 22 amino acid peptide of endothelial cell
origin and is structurally related to atrial natriuretic peptide but genetically distinct, and possesses vasoactive and
antimitogenic activity; vasonatrin peptide (VNP) which is a chimera of atrial natriuretic peptide (ANP) and C-type natriuretic
peptide (CNP) and comprises 27 amino acids; thrombin; endothelium-derived relaxing factor (EDRF); neutral endopeptidase 1 (NEP-1);
competitive inhibitors to EDRF, including, for example, NG-monomethyl-L-arginine (L-NMMA); potassium channel antagonists, such as
charybdotoxin and glibenclamide; antiheart antibodies, which may be identified in patients with idiopathic dilated cardiomyopathy
but which preferably do not elicit cytolysis in the myocardium; antibodies directed against the adenine nucleotide translocator,
the branched-chain keto acid dehydrogenase or cardiac myosin; specific antagonists for the endothelin-A receptor, which may be
referred to as BQ-123; and antibodies to the angiotensin II receptor.

17. Document ID: US 5356775 A

L10: Entry 17 of 19

File: USPT

Oct 18, 1994

US-PAT-NO: 5356775
DOCUMENT-IDENTIFIER: US 5356775 A

TITLE: Primary structure for functional expression from complementary DNA of a mammalian ATP-sensitive potassium channel

DATE-ISSUED: October 18, 1994

US-CL-CURRENT: 435/6; 435/252.3, 435/320.1, 435/69.1, 536/23.5

APPL-NO: 7/ 921178
DATE FILED: July 29, 1992
IN: Hebert; Steven C., Ho; Kevin

AB: This invention is directed to the cloning of the gene which encodes an ATP-sensitive K.sup.+ channel in
rat outer medulla cells, isolated cDNA sequences which encode said ATP-sensitive K.sup.+ channels, isolated proteins
produced by said cDNA sequences, and agents capable of binding to said proteins. Further included in the invention
are methods for identifying other members of the family of ATP-sensitive potassium channels (the ROMK1 family of
channel proteins), identifying, isolating, and cloning the genes which encode ROMK1 associated polypeptides,
identifying agents capable of binding to other members of the family, modulating expression of said family of
ATP-sensitive potassium channels, and modulating the activity of said family of ATP-sensitive potassium channels.
Additionally, included in the invention are methods for identifying drugs which function as K.sub.ATP channel
openers and K.sub.ATP channel closers.

L10: Entry 17 of 19

File: USPT

Oct 18, 1994

DOCUMENT-IDENTIFIER: US 5356775 A
TITLE: Primary structure for functional expression from complementary DNA of a mammalian ATP-sensitive potassium channel

Detailed Description Paragraph Right (92):
On the other hand, the loss of K.sub. ATP channel activity in isolated membrane patches (channel rundown) is at least partially reversed by MgATP but not by nonhydrolyzable analogues which suggests a role for phosphorylation in maintaining channel activity (Ribalet et al., J. Gen. Physiol. 94:693 (1989); Takano et al., Am. J. Physiol. 258:H45 (1990); Fiondlay, I., Pflugers Arch. 410:313 (1987)). Protein kinases A and C (Ribalet et at., J. Gen. Physiol. 94:693 (1989); Wolheim et at., EMBO J. 7:2443 (1988); De Weille et al., Proc. Natl. Acad. Sci. USA 86:2971 (1989)) have been found to modulate K.sub.ATP channel activity in different cell types. Likewise, the regulation of channel activity by PKA, PKC, and endogenous protein kinases has been demonstrated for both delayed rectifier (Walsh et al., Science 242:67 (1988); Rehem et al., Biochemistry 28:6455 (1989); Busch et al., Science 255:1705 (1992)) and Ca.sup.2+ -activated potassium channels (Reinhart et al., J. Neurosci. 11:1627 (1991); Chung et al., Science 253:560 (1991)). The presence of several potential phosphorylation sites for protein kinases A and C near the putative ATP-binding site in the ROMK1 protein is therefore intriguing. Clustering of multiple phosphorylation sites to a specific cytoplasmic domain has been noted in the .alpha. subunit of the rat brain Na.sup.+ channel (Rossie et at., J. Biol. Chem. 262:17530 (1987)), in subunits of some ligand-gated ion channels (e.g., muscle and neuronal acetylcholine receptors (AChR), .gamma.-aminobutyric acid (GABA) and glycine receptors) (Swope et al., FASEB J. 6:2514 (1992)), and in the R domain of the cFTR protein (Riordan et al., Science 245:1066 (1989)). In ROMK1, the apparent close association of phosphorylation sites and the putative nucleotide-binding sites raises the possibility that both types of sites may exert an effect on or participate in a common mechanism affecting channel opening and closure. In the "ball and chain" model of inactivation initially proposed for NA.sup.+ channels (Armstrong et al., J. Gen. Physiol. 70:567 (1977)), the movement of a cytoplasmic domain results in the occlusion of the ion channel pore and therefore channel inactivation. Such a mechanism has been demonstrated in Shaker channels involving an N-terminal segment consisting of a cluster of positively-charged amino acids and a hydrophobic domain (Hoshi et al., Science 250:533 (1990); Zagbotta et al., Science 250:568 (1990)); an analogous role has been proposed for the highly charged R domain of the CFTR protein (Riordan et al., Science 245:1066 (1989)). The finding that cytoplasmic proteolytic treatment of Shaker and NA.sup.+ channels either disrupts or slows inactivation is therefore consistent with the role of a cytoplasmic domain in channel inactivation. Similarly in pancreatic .beta.-cell K.sub.ATP channels, internal trypsin treatment results in an increase in channel activity, as well as, a reduction in sensitivity to inhibition by ATP (Trube et al., in Secretion and Its Control, G. S. Oxford and C. M. Armstrong, Eds. (Rockefeller University Press, New York, 1989), vol. 44, pp. 84-95.). A potential candidate for such an inactivation domain in ROMK1 is the highly-charged cytoplasmic segment following M2 (residues 181-232) which contains the putative phosphorylation sites and P-loop. Other possibilities include the N-terminal (residues 1-57) and C-terminal (residues 331-391) segments in which 35% and 39%, respectively, of the amino acids are charged. All three clusters of potential trypsin sites in ROMK1 overlap these three amino acid segments. The knowledge of the ROMK1 amino acid sequence enables the design of experiments which address these issues and many others.

18. Document ID: WO 9718332 A1

L10: Entry 18 of 19

File: EPAB

May 22, 1997

PUB-NO: WO009718332A1
DOCUMENT-IDENTIFIER: WO 9718332 A1
TITLE: REGULATION OF CANCER-CAUSING TYROSINE KINASES BY POTASSIUM ION CONDUCTANCE

PUBN-DATE: May 22, 1997

INT-CL (IPC): C12 Q 1/68; C07 K 14/00; A61 K 38/18; A61 K 38/43
EUR-CL (EPC): A61K031/505; A61K031/415, A61K038/15 , C07K014/705

APPL-NO: US09618304
APPL-DATE: November 12, 1996

PRIORITY-DATA: US55650495A (November 13, 1995)
IN: HOLMES, TODD C, LEVITAN, IRWIN B

AB: The present invention provides a method for treating protein tyrosine kinase-mediated conditions by
administering to a mammal an agent that increases cell membrane potassium ion conductance. Protein tyrosine
kinase-mediated conditions include psoriasis, atherosclerosis and cancers. In one embodiment the agent is a compound
that activates endogenous potassium ion channels. In a second embodiment, the agent is a compound which reacts with
potassium ion to form a membrane-permeant complex. In a preferred

embodiment, the agent is a potassium ion channel
protein administered via incorporation of a DNA molecule encoding the protein into the cellular genome. The present
invention also includes a pharmaceutical composition comprising a vector containing a gene encoding a potassium ion
channel protein in a form suitable for administration to a mammal. In yet another embodiment, the present invention
includes a method or assay for determining the activity of compounds which are potential potassium ion channel
activators or inactivators. The present invention also comprises a method for determining the virulence of cancerous
cells by measuring the potassium ion conductance of the cells.

L10: Entry 18 of 19 File: EPAB

May 22, 1997

DOCUMENT-IDENTIFIER: WO 9718332 A1
TITLE: REGULATION OF CANCER-CAUSING TYROSINE KINASES BY POTASSIUM ION CONDUCTANCE

Abstract (1):
The present invention provides a method for treating protein tyrosine kinase-mediated conditions by administering to a mammal an agent that increases cell membrane potassium ion conductance. Protein tyrosine kinase-mediated conditions include psoriasis, atherosclerosis and cancers. In one embodiment the agent is a compound that activates endogenous potassium ion channels. In a second embodiment, the agent is a compound which reacts with potassium ion to form a membrane-permeant complex. In a preferred embodiment, the agent is a potassium ion channel protein administered via incorporation of a DNA molecule encoding the protein into the cellular genome. The present invention also includes a pharmaceutical composition comprising a vector containing a gene encoding a potassium ion channel protein in a form suitable for administration to a mammal. In yet another embodiment, the present invention includes a method or assay for determining the activity of compounds which are potential potassium ion channel activators or inactivators. The present invention also comprises a method for determining the virulence of cancerous cells by measuring the potassium ion conductance of the cells.

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20020025949 A1	February 28, 2002		000	A61K031/66
WO 9840055 A2	September 17, 1998	E	089	A61K031/00
AU 9864923 A	September 29, 1998		000	A61K031/00
EP 969823 A2	January 12, 2000	E	000	A61K031/00
NZ 337849 A	June 29, 2001		000	A61K031/00
US 6306909 B1	October 23, 2001		000	A61K031/195
JP 2001515483 W	September 18, 2001		118	A61K045/00
AU 200195161 A	January 24, 2002		000	A61K031/00

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US20020025949A1	March 12, 1997	1997US-041140P	Provisional
US20020025949A1	February 3, 1998	1998US-073536P	Provisional
US20020025949A1	March 11, 1998	1998US-0041371	Div ex
US20020025949A1	August 16, 2001	2001US-0932676	US 6306909 Div ex
WO 9840055A2	March 12, 1998	1998WO-CA00244	AU 9864923A

19. Document ID: US 20020025949 A1, WO 9840055 A2, AU 9864923 A, EP 969823 A2, NZ 337849 A, US 6306909 B1, JP 2001515483 W, AU
200195161 A

L10: Entry 19 of 19 File: DWPI

Feb 28, 2002

DERWENT-ACC-NO: 1998-506461
DERWENT-WEEK: 200220

TITLE: Inhibiting epileptogenesis - comprising administration of agent which modulates process in pathway associated with epileptogenesis

PRIORITY-DATA: 1998US-073536P (February 3, 1998), 1997US-041140P (March 12, 1997), 1998US-0041371 (March 11, 1998), 2001AU-0095161 (November 29, 2001), 2001US-0932676 (August 16, 2001)

	March 12, 1998		
		1998AU-0064923	
AU 9864923A			
		WO 9840055	
			Based on
EP 969823A2			
	March 12, 1998		
		1998EP-0910555	
EP 969823A2			
	March 12, 1998		
		1998WO-CA00244	
EP 969823A2			
		WO 9840055	
			Based on
NZ 337849A			
	March 12, 1998		
		1998NZ-0337849	
NZ 337849A			
	March 12, 1998		
		1998WO-CA00244	
NZ 337849A			
		NZ 510633	
			Div in
NZ 337849A			
		WO 9840055	
			Based on
US 6306909B1			
	March 12, 1997		
		1997US-041140P	
			Provisional
US 6306909B1			
	February 3, 1998		
		1998US-073536P	
			Provisional
US 6306909B1			
	March 11, 1998		
		1998US-0041371	
JP2001515483W			
	March 12, 1998		
		1998JP-0539010	
JP2001515483W			
	March 12, 1998		
		1998WO-CA00244	
JP2001515483W			
		WO 9840055	
			Based on
AU 200195161A			
	March 12, 1998		
		1998AU-0064923	
			Div ex
AU 200195161A			
	November 29, 2001		
		2001AU-0095161	

INT-CL (IPC): A61 K 31/00; A61 K 31/195; A61 K 31/197; A61 K 31/216; A61 K 31/495; A61 K 31/505; A61 K 31/66; A61 K 38/00; A61 K 45/00; A61 P 25/08; C07 C 229/00; C07 C 229/28; C07 C 229/34; C07 C 229/42; C07 C 233/54; C07 D 239/54; C07 D 241/08

IN: CARRAN, J R, MILNE, P H, TAN, C Y K, WEAVER, D F, WEAVER, D E

AB: Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway
associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises
administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine;
(2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at
physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl,
alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or
aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10 alkyl, aryl, 1-30 alkylcarbonyl,
arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl
(optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl,
alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl,
arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4
and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a
method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or
calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from
NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding,
NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4)
compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an
iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group., USE - The method is used for treating -
neurological conditions comprising stroke, Alzheimer's disease, cancer and neurodegenerative disease. The agent is
administered orally and is then transported into the nervous system of the subject by an active transport shuttle
mechanism., Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway
associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises
administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine;
(2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at
physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl,
alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or
aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10 alkyl, aryl, 1-30 alkylcarbonyl,
arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl
(optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl,
alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl,
arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4
and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a
method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or
calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from
NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding,
NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4)
compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an
iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group., USE - The method is used for treating
neurological conditions comprising stroke, Alzheimer's disease, cancer and neurodegenerative disease. The agent is
administered orally and is then transported into the nervous system of the subject by an active transport shuttle
mechanism., Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway
associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises
administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine;
(2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at
physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl,
alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or
aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10

alkyl, aryl, 1-30 alkylcarbonyl,
arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl
(optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl,
alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl,
arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4
and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a
method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or
calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from
NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding,
NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4)
compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an
iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group., USE - The method is used for treating
neurological conditions comprising stroke, Alzheimer's disease, cancer and neurodegenerative disease. The agent is
administered orally and is then transported into the nervous system of the subject by an active transport shuttle
mechanism.

L10: Entry 19 of 19

File: DWPI

Feb 28, 2002

DERWENT-ACC-NO: 1998-506461
DERWENT-WEEK: 200220

TITLE: Inhibiting epileptogenesis - comprising administration of agent which modulates process in pathway associated with epileptogenesis

Basic Abstract Text:
Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine; (2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10 alkyl, aryl, 1-30 alkylcarbonyl, arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl (optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4 and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding, NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4) compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group.

Equivalent Abstract Text:
Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine; (2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10 alkyl, aryl, 1-30 alkylcarbonyl, arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl (optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4 and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding, NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4) compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group.

Basic Abstract Text (1):
Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine; (2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl,

alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10 alkyl, aryl, 1-30 alkylcarbonyl, arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl (optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4 and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding, NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4) compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group.

Equivalent Abstract Text (1):
Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine; (2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10 alkyl, aryl, 1-30 alkylcarbonyl, arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl (optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4 and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding, NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4) compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group.

Equivalent Abstract Text (3):
Inhibiting epileptogenesis comprises administration of an agent which modulates a process in a pathway associated with epileptogenesis. Also claimed are: (1) a method of inhibiting a convulsive disorder which comprises administration of a beta -amino anionic compound with the proviso that the compound is not beta -alanine or taurine; (2) compounds of formula (I) and dioxapiperazine compounds of formula (III) and their salts: A = an anionic group at physiological pH; R1 = alkyl, alkenyl, alkynyl, alkyl, aryl, alkoxy, aryloxy, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, OH, CN, halo, carboxyl, alkoxycarbonyloxy, aryloxycarbonyloxy or aminocarbonyl; R2, R3 = H, 1-30 alkyl, 2-30 alkenyl, 2-30 alkynyl, 4-10 alkyl, aryl, 1-30 alkylcarbonyl, arylcarbonyl, 1-30 alkoxycarbonyl or aryloxycarbonyl, or NR2R3 = optionally substituted 3-7 membered heterocyclyl (optionally substituted); Ar = optionally substituted aryl; R6, R6' = H, alkyl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl or aryloxycarbonyl; R7 = H, alkyl, mercaptoalkyl, alkenyl, alkynyl, alkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, CN, carboxyl, alkoxycarbonyl, aryloxycarbonyl or (CH2)n-Y; n = 1-4 and Y = H, thiazolyl, triazolyl or imidazolyl, provided that if Ar = phenyl, R7 is not H, methyl or phenyl; (3) a method for inhibiting a convulsive disorder which comprises administration of an agent which: (a) blocks sodium or calcium ion channels or opens potassium or chloride ion channels; and (b) has at least one activity selected from NMDA receptor antagonism, augmentation of endogenous GABA inhibition, calcium binding, iron binding, zinc binding, NO synthase inhibition and antioxidant activity, so that epileptogenesis and ictogenesis are inhibited and (4) compounds of formula (III) in which R6' = an antioxidant group, an NMDA antagonist, an NO synthase inhibitor, an iron chelator group, a Ca(II) chelator group or a Zn(II) chelator group.

1. Document ID: US 20020034781 A1

L1: Entry 1 of 3

File: PGPB

Mar 21, 2002

PGPUB-DOCUMENT-NUMBER: 20020034781
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020034781 A1

TITLE: 12303, a novel human TWIK molecule and uses thereof

PUBLICATION-DATE: March 21, 2002

US-CL-CURRENT: 435/69.1; 435/325, 435/6, 435/7.1, 530/350, 536/23.5

APPL-NO: 09/ 828035
DATE FILED: April 6, 2001

RELATED-US-APPL-DATA:
RLAN
RLFD
RLPC
RLKC
RLAC
60195734
Apr 7, 2000
US

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/195,734, filed on Apr. 7, 2000, incorporated herein in its entirety by reference.

IN: Glucksmann, Maria Alexandra

AB: The invention provides isolated nucleic acids molecules, designated TWIK-8 nucleic acid molecules, which encode novel TWIK-related potassium channel subunit molecules. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing TWIK-8 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a TWIK-8 gene has been introduced or disrupted. The invention still further provides isolated TWIK-8 proteins, fusion proteins, antigenic peptides and anti-TWIK-8 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

L1: Entry 1 of 3

File: PGPB

Mar 21, 2002

DOCUMENT-IDENTIFIER: US 20020034781 A1
TITLE: 12303, a novel human TWIK molecule and uses thereof

Summary of Invention Paragraph (13):
[0014] Another aspect of this invention features isolated or recombinant TWIK-8 proteins and polypeptides. In one embodiment, an isolated TWIK-8 protein includes at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain.

Summary of Invention Paragraph (14):
[0015] In a preferred embodiment, a TWIK-8 protein includes at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more iden amino acid sequence of SEQ ID NO: 2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____ .

Summary of Invention Paragraph (15):
[0016] In another preferred embodiment, a TWIK-8 protein includes one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and has a TWIK-8 activity (as described herein).

Summary of Invention Paragraph (16):
[0017] In yet another preferred embodiment, a TWIK-8 protein includes one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 3.

Brief Description of Drawings Paragraph (5):
[0032] FIG. 5 depicts the results of a search performed against the ProDom database which identified the presence of a "TRAAK potassium channel domain", a "potassium channel protein domain", a "voltage-gated potassium channel domain", an "outward-rectifier TOK1 potassium channel domain", and a "potassium channel subunit domain" in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2).

Detail Description Paragraph (19):
[0051] In another embodiment, a TWIK-8 molecule of the present invention is identified based on the presence of an "outward-rectifier TOK1 potassium channel domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "outward-rectifier TOK1 potassium channel domain" includes a protein domain having an amino acid sequence of about 25-100 amino acid residues and having a bit score for the alignment of the sequence to the outward-rectifier TOK1 potassium channel domain of at least 70. Preferably, an outward-rectifier TOK1 potassium channel domain includes at least about 40-75, or more preferably about 56 amino acid residues, and has a bit score for the alignment of the sequence to the outward-rectifier TOK1 potassium channel domain of at least 20, 30, 40, 50, 60, or higher. The outward-rectifier TOK1 potassium channel domain has been assigned ProDom entry 32818. To identify the presence of an outward-rectifier TOK1 potassium channel domain in a TWIK-8 protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein domains (e.g., the ProDom database) using the default parameters (available at http://www.toulouse.inra.fr/prodom.html). A search was performed against the ProDom database resulting in the identification of an outward-rectifier TOK1 potassium channel domain in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2) at about residues 215-270 of SEQ ID NO: 2. The results of the search are set forth in FIG. 5G.

Detail Description Paragraph (23):
[0055] Accordingly, another embodiment of the invention features isolated TWIK-8 proteins and polypeptides having a TWIK-8 activity. Preferred proteins are TWIK-8 proteins having at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel

domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and, preferably, a TWIK-8 activity.

Detail Description Paragraph (24):
[0056] Additional preferred proteins have at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 3.

Detail Description Paragraph (71):
[0103] In one embodiment, a biologically active portion of a TWIK-8 protein comprises at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain. It is to be understood that a preferred biologically active portion of a TWIK-8 protein of the present invention may contain at least one transmembrane domain and one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native TWIK-8 protein.

Detail Description Paragraph (257):
[0289] A search was also performed against the ProDom database (FIG. 5), resulting in the identification of "TRAAK potassium channel domains" from about residues 50-104 (score=175), 175-199 (score=115), and 288-382 (score=135); a "potassium channel protein domain" from about residues 99-153 (score=101); a "voltage-gated potassium channel domain" from about residues 102-168 (score=115); an "outward-rectifier TOK1 potassium channel domain" from about residues 215-270 (score=70); and a "potassium channel subunit domain" from about residues 216-287 (score=156) in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2).

2. Document ID: DE 19953478 A1

L1: Entry 2 of 3 File: DWPI

Oct 11, 2001

DERWENT-ACC-NO: 2001-603577
DERWENT-WEEK: 200169
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TITLE: Genetically modified yeast lacking endogenous potassium transport activity, useful for identifying e.g. antiarrhythmic agents, includes a functional human potassium channel

PRIORITY-DATA: 1999DE-1053478 (November 6, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
DE 19953478 A1	October 11, 2001		040	C12N001/19

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO
DE 19953478A1	November 6, 1999	1999DE-1053478

DESCRIPTOR

INT-CL (IPC): C12 N 1/19; C12 N 15/81; C12 Q 1/00
IN: LICHTENBERG-FRATE, H, LUDWIG, J

AB: NOVELTY - Genetically modified Saccharomyces cerevisiae (A) in which (i) the endogenous potassium-translocation systems (TRK1, TRK2 and TOK1) are specifically deleted and (ii) the human erg potassium ion channel (HERG) is stably integrated and expressed, is new., DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:, S. cerevisiae mutants with defects in potassium uptake as a result of mutation of the TRK1/2 and TOK1 genes, by introducing one or more selection markers (for auxotrophy or resistance);, (1) stably integrating human potassium ion channel genes for expression in the genome of a S. cerevisiae host that lacks functional TRK1/2 and TOK1; and, (2) identifying specific modulators of HERG., ACTIVITY - Antiarrhythmic; antiinflammatory., MECHANISM OF ACTION - Blocking or activating potassium channels., USE - (A) are used for identifying specific modulators of HERG, potentially useful as antiarrhythmic, antifibrillatory and antiinflammatory agents., ADVANTAGE - (A) are easier to culture than animals cells normally used; are genetically well characterized and are suitable for screening substance libraries in microtiter plates.

L1: Entry 2 of 3 File: DWPI

Oct 11, 2001

DERWENT-ACC-NO: 2001-603577
DERWENT-WEEK: 200169
COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: Genetically modified yeast lacking endogenous potassium transport activity, useful for identifying e.g. antiarrhythmic agents, includes a functional human potassium channel

Basi c Abstract Text:
NOVELTY - Genetically modified Saccharomyces cerevisiae (A) in which (i) the endogenous potassium-translocation systems (TRK1, TRK2 and TOK1) are specifically deleted and (ii) the human erg potassium ion channel (HERG) is stably integrated and expressed, is new.

Basic Abstract Text:
S. cerevisiae mutants with defects in potassium uptake as a result of mutation of the TRK1/2 and TOK1 genes, by introducing one or more selection markers (for auxotrophy or resistance);

Basic Abstract Text:
(1) stably integrating human potassium ion channel genes for expression in the genome of a S. cerevisiae host that lacks
functional TRK1/2 and TOK1; and

Basic Abstract Text (1):
NOVELTY - Genetically modified Saccharomyces cerevisiae (A) in which (i) the endogenous potassium-translocation systems (TRK1,
TRK2 and TOK1) are specifically deleted and (ii) the human erg potassium ion channel (HERG) is stably integrated and expressed, is
new.

Basic Abstract Text (3):
S. cerevisiae mutants with defects in potassium uptake as a result of mutation of the TRK1/2 and TOK1 genes, by introducing one or
more selection markers (for auxotrophy or resistance);

Basic Abstract Text (4):
(1) stably integrating human potassium ion channel genes for expression in the genome of a S. cerevisiae host that lacks
functional TRK1/2 and TOK1; and

3. Document ID: AU 200140424 A, WO 200151516 A2, DE 10001230 A1

L1: Entry 3 of 3 File: DWPI Jul 24, 2001

DERWENT-ACC-NO: 2001-442135
DERWENT-WEEK: 200166

TITLE: Identifying inhibitors and activators of eukaryotic potassium channels, for use as therapeutic agents, comprises using a transformed yeast cell that does not express endogenous channels

PRIORITY-DATA: 2000DE-1001230 (January 13, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200140424 A	July 24, 2001		000	C07K014/005
WO 200151516 A2	July 19, 2001	G	078	C07K014/005
DE 10001230 A1	August 2, 2001		000	C07K014/005

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO
AU 200140424A	January 15, 2001	2001AU-0040424
WO 200151516 A2	January 15, 2001	2001WO-DE00134
DE 10001230A1	January 13, 2000	2000DE-1001230

INT-CL (IPC): A61 K 38/16; C07 K 14/005
IN: GISSMANN, L, MICHEL, N, MUELLER, M, OSEN, W, ZENTGRAF, H

AB: NOVELTY - Identifying inhibitors (A) or activators (B) of a eukaryotic potassium channel by measuring the
effect of a test compound on a mutant Saccharomyces cerevisiae that:, (i) does not express the endogenous potassium
channels TRK1, TRK2 and TOK1; but, (ii) does express a heterologous eukaryotic potassium channel (I)., DETAILED
DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:, (a) mutant S. cerevisiae cell that does not
express the specified endogenous channels;, (b) producing cells of (a) by knockout of the RTK1, TRK2 and TOK1
genes;, (c) identifying (B) by measuring the effect of test compounds on the mutant S. cerevisiae in presence of a
known inhibitor;, (d) a test kit containing the cells of (a); and, (e) preparation of a pharmaceutical containing
(A) or (B)., ACTIVITY - None given., MECHANISM OF ACTION - Potassium channel inhibitor or activator., USE - The
method is used to identify substances that inhibit/activate human potassium channels selectively, potentially useful
as therapeutic agents, and also to detect toxic compounds., ADVANTAGE - The method is well suited for automation and
for performing many analyzes in parallel.

L1: Entry 3 of 3 File: DWPI Jul 24, 2001

DERWENT-ACC-NO: 2001-442135
DERWENT-WEEK: 200166
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TITLE: Identifying inhibitors and activators of eukaryotic potassium channels, for use as therapeutic agents, comprises using a transformed yeast cell that does not express endogenous channels

Basic Abstract Text:
(i) does not express the endogenous potassium channels TRK1, TRK2 and TOK1; but

Basic Abstract Text (2):
(i) does not express the endogenous potassium channels TRK1, TRK2 and TOK1; but